THE COMPARATIVE ENHANCEMENT OF THE BASAL METABOLISM AND OF THE ENDOGENOUS NITROGEN METABOLISM OF ALBINO RATS IN EXPERIMENTAL HYPERTHYROIDISM

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The demonstration by Terroine and Sorg-Matter ('37) and by Smuts ('35) that there is a relationship approaching constancy between the minimum endogenous nitrogen metabolism and the basal metabolism of energy among young adult animals of many species is a most important contribution to science, with important practical applications. Since the endogenous loss of nitrogen in the urine is the predominant component among the factors determining the maintenance requirement for protein, the constancy of this ratio among adult animals means that the maintenance requirement for protein varies with body size in a manner similar to the basal metabolism, i.e., in proportion to body surface (or to some fractional power of the body weight), rather than to body weight itself as is quite commonly supposed. Evidence, direct and auxiliary, supporting this principle has been cited by Smuts. In later experiments he has shown that the endogenous nitrogen metabolism of sheep (Smuts and Marais, '39) and of pigs (Du Toit and Smuts, '41) varies with body weight in much the same fashion as the basal metabolism.

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Smuts' ('35) experiments on mice, rats, guinea pigs, rabbits and pigs revealed the remarkably constant ratio of endogenous urinary nitrogen to basal heat of 2 mg per Cal. for adult, or near adult, animals, mostly males. Ratios recently reported for adult human subjects are considerably lower than this, averaging 1.42 mg per Cal. for 9 women (Bricker, Mitchell and Kinsman, '45), 1.41 mg per Cal. for 5 men (Murlin et al., '46) and 1.19 mg per Cal. for 6 women and 1.32 mg per Cal. for 7 men (Hawley et al., '48). Bricker, Mitchell and Kinsman discuss the possibility of a sex difference affecting the ratio and cite supporting evidence from other investigations. The University of Rochester group are frankly skeptical of the existence of any significant relationship between the endogenous nitrogen of the urine and the basal heat. In the words of the authors of the 1946 report referred to above: "It seems incongruous that one phase of (protein) maintenance metabolism which cannot be attained inside a month should be so definitely ('rigorously' is the term used by Terroine and Sorg-Matter) proportional to another phase (energy) of maintenance metabolism which can be attained in 15 hours." It should be noted that the statement by these workers concerning the time required to attain the minimum endogenous metabolism is contrary to their own experience. It may be noted further that the two experiments reported from the Murlin group were carried out in the summer months, the later one during "severely hot, humid weather." The losses of nitrogen in the sweat were not measured, were probably considerable, and quite probably diminished the urinary nitrogen on a nitrogen-free diet, since urea and ammonia account for over 50% of the nitrogen of sweat (Cuthbertson and Guthrie, '31). Sweat also contains creatinine (Mosher, '33; Mickelsen and Keys, '43), the urinary constituent uniquely characteristic of the endogenous catabolism. We have found that the summertime is a poor season in which to carry out accurate nitrogen, calcium, iron or iodine balance experiments by the usual methods on humans, because of the considerable losses of these elements in the sweat.

Nevertheless, experimental work subsequent to Smuts' report indicates clearly that the ratio of the minimum endogenous urinary nitrogen to basal heat is subject to disturbance in animals of different ages (Ashworth, '35; Ashworth and Cowgill, '38a; Treichler, '39), after various periods of body nitrogen depletion (Murlin et al., '46), and, in the rat at least, by the plane of nutrition (Treichler and Mitchell, '41). In hypophysectomized and thyroidectomized rats, according to Ashworth and Cowgill ('38b), the basal metabolism is depressed with no constant or appreciable effect on the endogenous urinary nitrogen: hence, the ratio is raised. However, the authors question whether the minimum endogenous level of urinary nitrogen had been reached in these experiments, especially in the thyroidectomized animals. In the experience of Nagornyi and Golubitskaya ('41), thyroidectomy lowers the endogenous nitrogen metabolism of rats of various ages.

The purpose of the experiments to be reported in this paper was to determine whether experimental hyperthyroidism in the rat, a condition known to elevate the basal metabolism readily, would elevate the rate of the endogenous nitrogen catabolism and in the same proportion. Hyperthyroidism has been shown to stimulate the total metabolism of nitrogen in the rat (Terroine and Babad, '39; Sure et al., '41), in the sheep (Blaxter, '48), and in the child (Johnston and Maroney, '39). On the assumption that iodinated casein does not disturb the ultilization of dietary protein, Blaxter ('48) estimates that the endogenous metabolism of nitrogen is markedly increased by this thyroxine preparation. In hyperthyroidism, the creatine output in the urine increases greatly, as shown by many authors, with either no effect on or a depression in the creatinine output. Although in the normal child there is a close relationship between the urinary creatinine and the basal metabolism, in the hyperthyroid child the creatinine output is not increased, in the experience of Talbot and his co-workers ('39).

The results of the experiments to be reported below demonstrate that experimental hyperthyroidism in adult rats, induced by feeding iodinated casein, augments both basal metabolism and endogenous nitrogen metabolism. For the dosages used, the two phases of the maintenance metabolism increase in a parallel fashion so that the ratio between the two is not appreciably disturbed.

TABLE 1

Plan of experiments, with food intakes and body weight changes of rats

PERIOD NO.	RATION	PERIOD FED	FOOD INTAKE	GAIN IN BODY WT.	DETERMINATIONS MADE
		days	*gm/day*	*gm/day*	
		Normal period			
I	Standard	42	13	+ 1.3	Basal heat
II	Low-N	17	16	+ 1.0	Urinary N
III	Standard	10	16	+ 1.6	Basal heat
		Hyperthyroid period [1]			
IV	Standard	14	16	— 0.3	Basal heat
V	Standard	10	19	0.0	Basal heat
VI	Low-N	16	19	— 1.5	Urinary N

[1] Fed 45 mg "Protamone" (iodinated casein) per rat per day. This dosage of protamone was found to increase the basal metabolism by about 70% in an exploratory experiment using identical rats.

MATERIALS AND METHODS

Plan of experiment

The experiments were carried out upon 7 adult male albino rats. They ranged in weight from 200 to 300 gm. Observations were made under normal conditions in periods I, II, and III (table 1) and after inducing hyperthyroidism by feeding iodinated casein [2] in periods IV, V and VI.

A basal metabolism measurement was made in periods I and III upon each of the 7 rats under the normal regime. Since it has been shown by Hamilton ('37, '39) and by Treich-

[2] Protamone obtained from the Cerophyl Laboratories, Inc. ,Kansas City, Missouri, through the courtesy of Dr. W. R. Graham, Jr.

ler and Mitchell ('41) that the basal metabolism of rats is markedly influenced by the previous caloric intake, all basal metabolism determinations were made after feeding the rats a constant amount of the experimental ration for about three weeks. In period II the endogenous urinary nitrogen was determined in a 7-day experiment following an adjustment period of the same length.

After the feeding of iodinated casein was started in period IV, several basal metabolism measurements were made on 4 rats to find out when the basal metabolism reaches a constant high level as a result of feeding the iodinated protein. Two or more basal metabolism tests were made on each of the 7 rats after the basal heat production reached its plateau. Since a slow loss of body weight was observed on iodinated casein feeding in period IV, the food intake in periods V and VI was raised from 16 to 19 gm per day per rat. The endogenous nitrogen was determined in period VI.

Basal metabolism

The basal metabolism of the rats was determined by the gravimetric method of Haldane (1892) as modified by Mitchell and Carman ('26a). The respiratory quotient was determined in experiments lasting two to three hours. Since the animals exhibited occasional activity during this period, the basal production of carbon dioxide was determined in shorter periods (about 30 min.) of complete muscular repose. The rats were fasted for at least 17 hours before the basal measurements were made. The temperature inside the respiration chamber was always within the range of thermal neutrality (28–33°C.) for fasting rats as observed by Swift ('44). In calculating the basal heat production, protein metabolism was not considered and total respiratory quotients were assumed to represent the non-protein respiratory quotients. The error introduced by such simplification rarely exceeds 2% (Smuts, '35, p. 412).

Experimental diets

Two experimental diets were employed (table 2); one, the "standard" diet, was used during all times except when the minimum endogenous urinary nitrogen was to be determined, and the other, the low-nitrogen diet, during periods II and VI. The former ration contained 9.7% and the latter 3.0% of whole egg protein. All rations were fed in amounts to maintain approximately the body weights of the rats.

TABLE 2

Percentage composition of experimental diets

INGREDIENTS	STANDARD RATION	LOW-N RATION
	%	%
Dried ether-extracted whole egg	13.0	4.0
Wood flock [1]	2.0	2.0
Amidex (dextrin)	31.0	32.0
Vitaminized starch [2]	5.0	5.0
Cerelose (glucose)	26.0	34.0
Sucrose	10.0	10.0
Salt mixture 446 [3]	4.0	4.0
Wheat germ oil	0.5	0.5
Cod liver oil	1.5	1.5
Lard	6.0	6.0
Sodium chloride	1.0	1.0
Per cent protein	9.7	3.0

[1] Obtained from Brown Company, Portland, Maine.

[2] Containing all of the known members of the B-complex execept biotin and folic acid.

[3] See Spector ('48).

The endogenous urinary nitrogen excretion

Mitchell and Carman ('26b, p. 200) have shown that whole egg protein at a level of 3 or 4% in the diet, while maintaining the appetite better, does not affect appreciably the output of urinary nirogen on a nitrogen-free diet. The collections of urine were made daily by the methods employed in this laboratory, the adequacy of which has been satisfactorily demonstrated (Smuts, '35, p. 416–17).

Methods of analysis

All urine collections were analyzed for total nitrogen by the Kjeldahl method, using mercury and potassium sulfate in the digestion mixture. Preformed creatinine was determined by the method described by Hawk, Oser and Summerson ('47). For total creatinine, a modification of the Folin method (Mitchell and Carman, '26b) was used; creatine was calculated by difference.

TABLE 3

Summary of metabolism data on the rats in normal periods

RAT NO.	BASAL HEAT PRODUCTION [1]	RESULTS OBTAINED IN PERIOD II			RATIO OF ENDOGENOUS N TO BASAL HEAT
		Gain in body wt.	Endogenous urinary nitrogen		
	cal./m²/day	*gm/day*	*mg/day*	*mg/m²/day* [2]	*mg/cal.*
3	953	+ 0.4	58.9	1614	1.69
4	946	+ 0.5	48.9	1385	1.46
6	786	+ 1.6	42.0	1138	1.45
7	873	+ 1.8	42.9	1205	1.38
8	908	+ 0.5	57.4	1612	1.77
9	927	+ 0.9	57.4	1568	1.69
10	943	+ 1.4	43.7	1190	1.26
Average	905			1387	1.53

[1] Data obtained in period III.

[2] See footnote 1, table 6.

EXPERIMENTAL RESULTS

The metabolic observations on the rats prior to the induction of hyperthyroidism are summarized in table 3. The minimum endogenous urinary nitrogen averaged 1387 mg and the basal metabolism 905 cal. per m^2 per day. The average ratio between the two is 1.53 mg per cal. This ratio is lower than that reported by Smuts ('35), i.e., 1.99, or that reported by Treichler and Mitchell ('41), i.e., 1.95 for adult rats on maintenance nutrition. The discrepancy may be due in part to the fact that the rats in period III, during which the basal

metabolism was measured, were receiving considerably more than a maintenance ration in anticipation of the higher energy requirements during the hyperthyroid condition that was to be imposed later. During this period they were gaining 1.6 gm per day on the average (table 1). On surfeit feeding, Treichler and Mitchell secured an average of 1.66 for adult male rats.

TABLE 4

Increase in the basal metabolism of the rats after different periods of feeding iodinated casein [1]

RAT NO.	PERIOD ON PROTAMONE	BASAL HEAT PRODUCTION				INCREASE IN HEAT PRODUCTION
		Normal value [2]	R.Q.	Hyperthyroid values	R.Q.	
	days	*cal./m²/day*		*cal./m²/day*		%
4	10	946	0.72	1631	0.74	72
	13			1723	0.72	82
	21			1622	0.76	71
	26			1697	0.74	79
	29			1657	0.74	75
6	9	786	0.73	1349	0.73	71
	23			1330	0.77	69
	28			1296	0.73	65
7	10	873	0.74	1512	0.75	73
	15			1865	0.75	114
	20			1916	0.78	119
8	5	908	0.70	1271	0.71	39
	18			1639	0.71	80
	22			1618	0.72	78

[1] "Protamone."

[2] Values obtained in period III.

In order to determine the time required for the rats to adjust themselves to the selected dosage of protamone (45 mg per rat per day), the basal heat production of 4 of the rats was determined from time to time for a period of three to 4 weeks after dosage started. The values obtained are presented in table 4. It appears that the basal metabolism is approximately stabilized after about three weeks of dosage.

TABLE 5

Metabolism data on the hyperthyroid rats

RAT NUMBER	BASAL HEAT PRODUCTION		CHANGE IN BODY WEIGHT	RESULTS OBTAINED IN PERIOD VI			RATIO OF ENDOGENOUS N TO BASAL HEAT	TOTAL CREATININE N OF ENDOGENOUS N	CREATINE FRACTION OF TOTAL CREATININE
				Endogenous urinary N					
		Increase over normal				Increase over normal			
	cal./m²/day	%	*gm/day*	*mg/day*	*mg/m²/day*	%	*mg/cal.*	%	%
3	1748	83	— 1.6	95.5	2652	64	1.52		37
4	1675	77	— 0.3	98.6	2961	114	1.77	6.5	25
6	1313	67	+ 0.1	97.0	2493	119	1.90	7.4	32
7	1890	116	— 0.4	85.7	2498	107	1.32	5.8	27
8	1628	79	— 0.4	88.7	2609	62	1.60	7.1	35
9	1731	86	— 0.8	91.3	2564	63	1.48	5.6	22
10	1753	86	— 1.1	94.1	2536	113	1.45	6.6	23
Averages	1677	85			2616	92	1.58	6.5	29

The complete metabolism data on the hyperthyroid rats, secured after stabilization at the dosage of iodinated casein used, will be found in table 5, which also affords comparisons with the observations secured on the rats prior to dosage. Due to the induced hyperthyroidism, the basal metabolism of the rats increased over a range of from 67 to 116%, averaging 85%. Simultaneously the minimum endogenous urinary nitrogen increased through a range of from 62 to 119%,

TABLE 6

Basal metaoblism of the rats at two different food intake levels

RAT NO.	AT LOWER LEVEL IN PERIOD I (13 GM FOOD/DAY PRIOR TO BASAL)			AT HIGHER LEVEL IN PERIOD III (16 GM FOOD/DAY PRIOR TO BASAL)			INCREASE IN BM AT HIGHER LEVELS OF FOOD INTAKE
	Body weight	Basal heat [1]	R.Q.	Body weight	Basal heat	R.Q.	
	gm	*cal./m²/day*		*gm*	*cal./m²/day*		%
3	266	911	0.70	288	953	0.76	+ 4.6
4	244	842	0.71	256	946	0.72	+ 12.3
6	258	785	0.64 [2]	293	786	0.73	+ 0.1
7	250	861	0.69 [2]	273	873	0.74	+ 1.4
8	254	867	0.74	263	908	0.70	+ 4.7
9	259	887	0.70	292	927	0.74	+ 4.5
10	261	857	0.72	292	943	0.73	+ 10.0
Average		858			905		+ 5.4

[1] Surface area of the rats was estimated according to the formula of Lee ('29) viz., $S_m^2 = 12.54\ W_{gm}^{0.66}$.

[2] The heat production in all periods for which R.Q.'s of less than 0.707 were obtained was computed by assuming a heat equivalent of a liter of oxygen of 4.686 cal.

averaging 92%. The ratio of endogenous urinary nitrogen to basal heat did not change significantly, the average of the hyperthyroid state being 1.58 and for the normal state 1.53.

Part of the average increase of 85% in the basal metabolism of the hyperthyroid rats may have been a result of the greater plane of nutrition on which they had been subsisting, since they received 19 gm of food daily as compared to 16 gm in the period of normal treatment. The effect on the basal metabolism of a 3 gm difference in daily food intake is shown in table

6. In all cases the metabolism was higher when the basal condition was established after a period of feeding 16 gm of food daily than after a period of feeding 13 gm. The differences ranged from 0.1 to 12.5% and averaged 5.4%. It may be concluded, therefore, that very little of the elevation in the metabolism of the hyperthyroid rats over the normal rate can be ascribed to the higher plane of pre-feeding.

Table 5 confirms many previous studies in hyperthyroidism by showing a marked creatinuria in the experimental animals. An average of 29% of the total urinary creatinine was present as creatine, a clear indication in itself of an accelerated endogenous nitrogen catabolism. The total creatinine nitrogen averaged 6.5% of the total urinary nitrogen, a value not greatly different from that reported by Smuts ('35) for normal rats, i.e., 7.1%.

The fact that thyroid-active material will increase the minimum endogenous nitrogen metabolism as well as the basal metabolism, and that the increases are approximately in the same proportion, is in harmony with the Terroine-Smuts principle that the two types of maintenance metabolism are related in magnitude, and that the protein requirements for maintenance, as well as the energy requirements, vary with the body surface more nearly than with the body weight, other determining factors remaining the same.

SUMMARY AND CONCLUSIONS

Metabolism experiments were performed on 7 adult male albino rats, first under normal conditions and then under conditions of hyperthyroidism induced by dosage with iodinated casein. The basal metabolism of energy and the output in the urine of nitrogen from endogenous sources only were determined under both normal and hyperthyroid conditions by the usual methods.

Both the basal metabolism and the minimum endogenous nitrogen metabolism are elevated 80 to 90% during hyperthyroidism of the intensity induced. The percentage of total

creatinine of the total endogenous urinary nitrogen is not greatly disturbed, although a marked creatinuria is induced.

The similar effect of thyroid-active material on the basal metabolism and on the minimum endogenous metabolism of nitrogen in the adult animal is in harmony with the Terroine-Smuts principle that the two are related causally and, under normal conditions, vary with body size in a parallel fashion, other determining factors remaining the same.

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ANALYTICAL AND EXPERIMENTAL STUDY OF THE EFFECTS OF INCREASED PROTEIN WITH LIBERAL CALCIUM AND RIBOFLAVIN INTAKES: COMPLETE LIFE CYCLES [1]

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ONE FIGURE

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Slonaker (several researches summarized in '39), in long term experiments with rats on diets containing 10, 14, 18, 22, and 26% protein, found 14% the most advantageous level. While no other work has superseded that of Slonaker, some other findings have raised the question of whether more protein could be utilized to advantage if more attention were given to liberality of intake of mineral elements and vitamins, especially calcium and riboflavin.

Advantage from extra protein thus fed might be detected either by analytical determination of higher levels of protein or riboflavin or both in the body, or by enhanced biochemical functioning as shown in growth, adult vitality, and length of life.

In connection with the use of diets consisting largely or mainly of wheat (or flour) and milk, with the rat as experimental animal, it was found by MacLeod ('26–'27), by Russell ('32), and by one of the present authors (H.L.C., unpublished

[1] Aided by grants from Swift and Company to Columbia University.

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data) that the addition of meat to these diets tended to increase growth and reproduction records. Individual differences, however, were such that averages for 15 females on each of two comparable diets proved insufficient for conclusive findings. Later it became possible for us to study the effects of increased protein with much larger numbers of animals, as described below.

Meanwhile, Slonaker ('39) reported upon the effect of different percentages of protein in the diets of 6 generations of rats. He observed (as in his earlier work) better results in rats fed a diet containing 14%, than in those fed 10, 18, 22, or 26% of protein in the air-dry food mixture. Repeatedly, Slonaker cites his finding of 14% as optimum: in one case he adds, "supplemented by a higher percentage of protein for rearing the young," i.e., during lactation. While his extensive observations thus showed a few indications that a protein content slightly higher or lower than 14% might in particular cases have given better results, it seemed evident that for the conditions of his experiments the optimum level of protein intake for lifelong well-being was certainly not over 16%. Experiments on growth alone may, of course, favor much higher levels of intake.

PURPOSE AND PLAN OF THE PRESENT WORK

The purpose of the experiments here reported was to ascertain whether a higher intake of protein than that which appeared optimum in Slonaker's work can be used to increased advantage if the experimental diet also contains liberal proportions of calcium, vitamin A, and riboflavin.

The starting point of these experiments was our laboratory diet 133, consisting of two-thirds ground whole wheat and one-third dried whole milk, with the addition of sodium chloride amounting to 2% of the weight of the wheat and enough calcium carbonate to give the food mixture a calcium content of 0.64%. The riboflavin content was approximately 7.2–7.7μg/gm; the protein, 16%; vitamin A value, 6 I.U./gm. In strict parallel was fed laboratory diet 180, which differed

from diet 133 only in that dry beef muscle protein was added to increase the protein content of the food mixture by one-fourth. This brought the total protein content to 20%, while the riboflavin content was within the above-given range of 7.2–7.7 μg/gm. The beef muscle protein [3] was thoroughly defatted and contained only insignificant amounts of anything other than protein and hygroscopic moisture.

Each of our series of experiments was begun by assembling strictly parallel "families" or "lots" by assignment of litter mates of the same sex and size to each of the two diets above described (diets 133 and 180). The initial age was 28 days. The sexes grew up together, usually three females and two males in a 12 × 15 inch cage, and the age at which each female gave birth to her first young was one of the objective criteria as to whether the difference in diet influenced the rate of development. Weights were recorded weekly. Each female, when she showed pregnancy, was placed in a separate cage and given opportunity to rear as many of her young as she could. As each litter reached the age of 28 days the mother was returned to her permanent cage and the young assigned either for analysis at predetermined ages or to the assembling of new breeding lots to carry the study through a second generation.

As the influence of an experimental diet is sometimes clearer in the second generation, it was desirable that some of these second generation lots be included in the investigation; but not so many as to reduce unduly the number of new samplings drawn from the long-established colony.

RESULTS OF ANALYSES OF EXPERIMENTAL ANIMALS FROM DIFFERENT DIETS AT PREDETERMINED AGES

The averages resulting from the analyses are summarized in tables 1 and 2, the figures in parentheses showing the number of cases entering into each tabulated average. Nitrogen

[3] The beef muscle protein was prepared in the research laboratories of Swift and Company and furnished us through the courtesy of Dr. H. E. Robinson, to whom we are greatly indebted for this cooperation.

(protein), phosphorus, and riboflavin were determined in the muscle and liver of rats from families on each of the diets at the ages of 30, 60, 90, 120, 150, and 180 days.

The muscles of the animals from each diet showed a slight upward trend of nitrogen (protein) and downward trend of riboflavin concentration during the age range covered by these analyses.

At none of the ages here studied did the nitrogen, phosphorus, or riboflavin concentration of muscle or liver appear to be significantly influenced by the difference in the protein content of these diets, although it is known that by means of

TABLE 1

Nitrogen and phosphorus content in per cent of moist muscle and moist liver

AGE	MOIST MUSCLE				MOIST LIVER			
	Nitrogen		Phosphorus		Nitrogen		Phosphorus	
	From diet 133	From diet 180	From diet 133	From diet 180	From diet 133	From diet 180	From diet 133	From diet 180
days	%	%	%	%	%	%	%	%
30	2.92(4)[1]	3.00(4)	0.244(4)	0.238(5)	2.62(4)	2.66(5)	0.269(4)	0.266(4)
60	3.23(3)	3.29(2)	0.229(3)	0.234(2)	3.01(3)	3.04(2)	0.253(2)	0.263(2)
90	3.26(5)	3.14(5)	0.242(5)	0.238(3)	2.98(5)	2.92(5)	0.281(5)	0.290(5)
120	3.34(4)	3.30(2)	0.255(4)	0.246(2)	3.09(4)	3.08(2)	0.296(4)	0.283(2)
150	3.44(3)	3.44(3)	0.235(3)	0.255(3)	3.04(3)	3.16(3)	0.277(3)	0.247(3)
180	3.26(3)	3.36(4)	0.224(3)	0.222(4)	2.75(3)	3.03(4)	0.262(3)	0.226(4)

[1] Numbers in parentheses indicate number of cases.

TABLE 2

Riboflavin in moist muscle and liver

AGE	MOIST MUSCLE		MOIST LIVER	
	From diet 133	From diet 180	From diet 133	From diet 180
days	*μg/gm*	*μg/gm*	*μg/gm*	*μg/gm*
30	4.1(65)[1]	4.2(58)	20.3(64)	19.9(57)
60	3.6(20)	3.4(15)	23.5(20)	25.2(15)
90	3.3(19)	3.4(15)	25.0(20)	23.7(15)
120	3.3(16)	3.1(14)	27.4(16)	25.2(15)
150	2.8(13)	3.1(14)	25.1(14)	22.8(14)
180	2.9(16)	2.9(14)	23.8(19)	22.5(15)

[1] Numbers in parentheses indicate number of cases.

larger differences of protein intake one may influence the concentrations of protein and riboflavin in body tissues (Sarrett, Klein, and Perlzweig, '42; Sherman and Ragan, '48).

The fact that the difference of protein intake here studied did not cause a measurable difference of concentration levels in the body makes especially noteworthy the further fact that this same difference of intake did have measurable effects upon some aspects of biochemical performance as recorded below and in table 3.

While the beef muscle protein kindly furnished us by Dr. Robinson [4] for these experiments was not a chemically pure substance, we see no reason to doubt that its significance here was simply with respect to its protein content. As explained in the above description of the diets, the increase of protein content (diet 180 over diet 133) was effected within a variation in riboflavin or any minor substance of only about one-twentieth of the amount concerned.

In a series of parallel experiments it was found that increasing by one-fourth the phosphorus and riboflavin contents of the diet had no measurable influence upon the growth of the body or upon the nitrogen, phosphorus, or riboflavin content of the tissues. Thus the phosphorus intake was not the limiting factor in the bodily retention of nitrogen, of phosphorus, or of riboflavin, the level of intake of each being presumably within the range of its optimum. Apparently the body's concentration of phosphorus remained somewhat more constant than that of nitrogen (protein) or riboflavin during the normal growth of the rat from the age of 30 to that of 180 days. While this growth undoubtedly involved an increased bodily amount (as distinguished from concentration) of a riboflavin-phosphate-protein compound or compounds, the effects of the increase of dietary protein in these experiments are to be interpreted as essentially protein effects not complicated by any limitation of phosphorus or of riboflavin.

Thus the figures in tables 1 and 2 show that increasing the protein content of the diet from 16% to 20% — an increase

[4] See footnote 3, p. 319.

TABLE 3

Biochemical performance on diets of different protein content

	ON DIET 133 (16% PROTEIN)	C.V.	ON DIET 180 (20% PROTEIN)	C.V.	DIFFERENCE
Gain in weight, 28th–56th days of age					
females (gm)	66.1 ± 0.61 (84)[1]	13	73.9 ± 0.78 (83)	14	7.8 ± 0.99
males (gm)	88.6 ± 1.45 (54)	18	106.2 ± 1.32 (55)	14	17.6 ± 1.96
Age of females at birth of first young (days)	101.9 ± 1.49 (82)	20	98.2 ± 1.21 (80)	17	3.7 ± 1.92
Duration of reproductive life of females (days)	297.0 ± 11.4 (84)	53	366.0 ± 10.5 (84)	40	69.0 ± 15.3
Young borne per female	34.0 ± 1.24 (84)	50	42.8 ± 1.39 (84)	45	8.8 ± 1.86
Young reared per female	23.3 ± 1.01 (84)	60	31.3 ± 1.27 (84)	56	8.0 ± 1.62
Per cent of young reared	68.6 ± 1.73 (82)	34	70.6 ± 1.75 (80)	33	2.0 ± 2.46
Aver. weight of young at 28 days (gm)	46.1 ± 0.09 (1957)	12	50.9 ± 0.05 (2626)	8	4.8 ± 0.10
Length of life					
males (days)	711.0 ± 10.5 (55)	16	728.0 ± 12.7 (55)	19	17.0 ± 16.4
females (days)	783.0 ± 16.2 (83)	28	824.0 ± 11.7 (83)	19	41.0 ± 20.0

[1] Numbers in parentheses indicate number of cases.

of one-fourth over the initial concentration in the food — did not measurably increase the protein, riboflavin, or phosphorus content of the tissues. Yet this difference of protein intake did measurably influence biochemical performance as shown below.

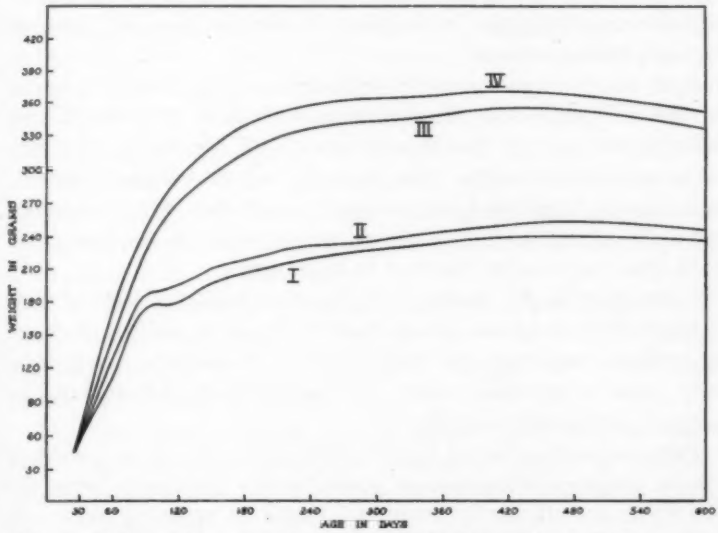

Fig. 1 Body weight curves of the rats, the further records of which are summarized in table 3: I — females on diet 133 (16% protein); II — females on diet 180 (20% protein); III — males on diet 133; IV — males on diet 180.

INFLUENCE OF THE LEVELS OF PROTEIN INTAKE UPON BIOCHEMICAL PERFORMANCE

The aspects of biochemical performance to which attention has here been especially directed because of their objectivity and measurability are growth, success in reproduction and rearing of the young, and length of life. The initial numbers of experimental animals on each of the two diets were 55 males and 84 females, of which latter no less than 80 (or 95%) of those on each diet reared young. The average data for the animals on each diet with respect to each of the criteria

here measured are summarized in table 3, from which it will be seen that notwithstanding the absence of measurable differences in body composition (tables 1 and 2) certain objective criteria of biochemical performance do show measurable effects of the difference in the protein content of the diet. All significant differences here found were in the direction of higher records made by animals from families on diets of higher protein content.

Both sexes unquestionably showed more rapid early growth on the diet with 20% than on that with 16% protein. These differences are for females 8 times and for males 9 times their probable errors. The animals on the higher protein diet also had higher body weights as adults. Weight curves for each sex on each diet are shown in figure 1, the irregular data of old age being omitted to save space.

Correspondingly, females on the diet with the higher percentage of protein bore their first young at an earlier average age. Here, however, the difference is relatively much less, only twice its probable error, so that by itself it would not be judged statistically significant.

Females on the diet of higher (20%) protein content showed longer average duration of reproductive life, and here the difference is sufficiently large (4.5 times its probable error) to be judged statistically significant.

The numbers of young borne and reared were also higher for the females on the diet with 20% than for those on that with 16% of protein, and here the differences (4.7 and 5.0 times their probable errors, respectively) are statistically significant in both cases.

The percentage of young reared was essentially the same for the families on the two diets.

The average weight of the young at the conventional separation age of 28 days is unquestionably greater in the families on the diet with 20% than in those on the ration with 16% of protein, the difference being 48 times its probable error.

On each diet the females lived longer than the males, as is usual in this colony.

Neither with the males nor with the females did the increased protein content of the diet significantly influence the length of life.

DISCUSSION

It is clear that raising the protein content of the diet by one-fourth, from an initial 16% to 20%, by addition of beef muscle protein to the air-dry food mixture increased the rate of growth and (less markedly) the adult size of the experimental animals without measurably increasing the concentration of protein, riboflavin, or phosphorus in the body tissues.

Yet the effect of the extra protein, while not demonstrable by direct analyses of the tissues, did measurably increase not only the growth and size of both sexes, but also the reproduction records of the females.

Here the long-term quantitative observation of the body's biochemical performance proved a more delicate method of finding the effects of the dietary change than did direct chemical analysis of tissue.

The fact that a higher protein intake level was used more advantageously here than in Slonaker's work is probably due to more liberal calcium, riboflavin, and vitamin A values in our basal diet.

How may one picture a chemical improvement not demonstrable by dircet chemical analyses? Three possible concepts suggest themselves, any or all of which may be true.

The concentration principle suggests that increased protein intake may raise the concentration of proteins or of some of their derivatives in the body by a margin too small to be found by present methods of analysis yet which, continuing for a lifetime, may measurably influence the record of biochemical performance.

A second possibility is that the flowing of a larger amount of protein through the metabolic process, even at an unchanged concentration level, may tend to raise the *tone* of the life process, possibly by interaction with other nutrients.

A third hypothesis is that the larger animal which tends to result from the higher protein intake may have some advantage of physical capacity over a smaller individual of the same strain and chemical composition. Comparison of the larger and smaller animals on the same diet indicates that the former show a slight advantage in their breeding records but no significant difference in length of life.

SUMMARY AND CONCLUSIONS

In view of recent advances in the biochemistry of calcium, riboflavin, and vitamin A, long-term experiments have been made (with rats) to test the effect of increasing the protein content of the diet from an initial 16% to 20% in the air-dry food mixture, the basal diet being liberal in its calcum, riboflavin and vitamin A content.

With this basal diet the increase of protein intake proved more advantageous in its long-term effects than had been the case in the corresponding work by Slonaker ('39). Animals receiving food containing 20% as contrasted with 16% of protein showed increased growth and attained a larger adult size and the females made higher reproduction records, though direct chemical analyses of body tissues showed no measurable change of concentration of protein, phosphorus, or riboflavin. Hypotheses relating to the greater precision of some long-term criteria than of direct chemical analysis by present-day methods are discussed briefly.

The moderate difference of protein intake here studied did not significantly influence the length of life or the percentage of young reared, though the higher protein diet did result in somewhat larger size in both sexes and in the rearing of more and larger young.

ACKNOWLEDGMENT

The executive guidance of Professor A. W. Thomas, and the efficient services of all who took part in the work, whether as research assistants or volunteers, are gratefully acknowledged.

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NINE ESSENTIAL AMINO ACIDS IN PURE VARIETIES OF WHEAT, BARLEY AND OATS[1]

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Available data (Baumgarten, Mather and Stone, '46; Block and Mitchell, '46; Schweigert, '47, '48) on the essential amino acid content of cereal grains are based largely on analyses done on limited numbers of samples of indefinite origin. Assay results for large numbers of samples of known origin and variety are required to provide information regarding variability in quality of grain protein and possible relationships between such factors as soil, climate, variety and species and the essential amino acid composition of cereal grain proteins. Results of microbiological assays conducted on 9 samples of wheat, barley and oats of known variety and origin are reported in the present paper. Assays were repeated a sufficient number of times to permit statistical analysis of the results.

EXPERIMENTAL

Assay material

The grains studied were Marquis wheat, Newal barley and Victory oats grown in 1945. Samples were selected to cover a wide range in protein content and to represent grains grown

[1] Part of the data in this paper was taken from a thesis submitted by William Lobay in partial fulfillment of the requirements for the M. Sc. degree. Financial assistance was received from the Committee on Agricultural Research Grants, University of Alberta, and from the National Research Council of Canada. Paper 263 of the Associate Committee on Grain Research.

under a variety of soil and climatic conditions. With a few exceptions they were obtained from University of Alberta test plots and from Dominion Illustration and Experimental Stations located in representative soil zones throughout the province of Alberta.

Assay methods

Nitrogen and dry matter. Nitrogen determinations were done on one gram samples by the Kjeldahl-Gunning-Arnold method (A.O.A.C., '45) using mercuric oxide as catalyst. Two or more determinations in duplicate were made on each sample. Dry matter was determined by heating finely ground samples in a gravity convection oven for 18 hours at 105°C.

Hydrolysates. Hydrolysates were prepared by heating one gram samples of finely ground grain in 25 ml of 2N hydrochloric acid in an autoclave for 10 hours at 15 pounds pressure. The results of preliminary tests on duration of hydrolysis showed that the level of α-amino nitrogen, as measured by the Van Slyke nitrous acid method, reached a maximum after 7 hours in the autoclave, and remained constant at intervals between 7 and 20 hours.

Amino acids. A minimum of two sets of paired hydrolysates of each sample were assayed microbiologically for 9 essential amino acids, using *Lactobacillus arabinosus* 17–5 for leucine, isoleucine, valine and phenylalanine; *Streptococcus faecalis* R for methionine, threonine, histidine and arginine; and *Leuconostoc mesenteroides* P-60 for lysine. The media used and the assay technique followed were the same as those employed by Riesen, Clandinin, Elvehjem and Cravens ('47).

RESULTS AND DISCUSSION

The results of assays done on 9 samples of Marquis wheat, Newal barley and Victory oats, expressed in terms of per cent of the total nitrogen of a sample contributed by the nitrogen of each of 9 essential amino acids, are summarized in table 1. Values listed for each sample and each amino acid represent the average of from three to 6 assays completed in duplicate.

Statistical tests for significance (Goulden, '39; Snedecor, '46) were applied to the original data when the mean values listed in table 1 suggested the possibility of differences between species or between samples of one variety of one species.

Sample differences within a variety of one species

Inspection of the data in table 1 shows that in all three grains some variation may exist in the per cent of total nitrogen contributed by the nitrogen of a given amino acid in different samples of one variety. This variability appears to be most marked for the amino acids lysine, arginine, valine and phenylalanine. *F* values, derived for variance due to samples within a variety of one species, were significant beyond the 1% point for lysine in all three grains, for arginine and valine in Marquis wheat and Newal barley, and for phenylalanine in Victory oats and Newal barley.

Samples in table 1 are listed in order of nitrogen content and it may be noted that in the wheat and barley samples there is a trend towards a decrease in per cent lysine nitrogen of total nitrogen with increasing levels of the latter. The values reported by Schweigert ('48) for lysine in 5 samples of wheat of different protein content also suggest the possibility of an inverse relationship between total protein and per cent lysine of protein. Regression coefficients were calculated for the present data and it was found that in the wheat samples lysine nitrogen as per cent of total nitrogen decreased 0.021% for each increase of 1% in total nitrogen, but neither this coefficient nor that of −0.019 derived for barley was found to be significant at the 5% point by the *t* test.

It is recognized (Anderson, '39–'41) that the total protein content of grains is affected by the conditions of soil and climate under which they are grown; for example, it is evident from table 1 that the samples grown in the gray-wooded soil zone contained less protein than those grown in the brown soil zones. The present data do not, however, demonstrate the existence of a significant correlation between soil zone and

TABLE 1

Per cent of total nitrogen contributed by 9 essential amino acids in grains of different protein content.
(Data are reported on a moisture-free basis)

SAMPLE NO.	SOIL ZONE [1]	TOTAL N	AMINO ACID NITROGEN FRACTION OF TOTAL NITROGEN									
			Leucine	Isoleucine	Valine	Phenylalanine	Methionine	Threonine	Histidine	Arginine	Lysine	Total
		%	%	%	%	%	%	%	%	%	%	%
Marquis wheat												
W- 3	G.	1.94	4.23	3.14	3.56	2.68	0.72	2.01	2.89	9.38	2.89	31.50
W- 4	D.B.	2.64	4.39	3.18	3.49	2.61	0.76	2.08	2.65	9.47	2.96	31.59
W- 2	Bl.	2.85	4.14	3.09	3.30	2.70	0.67	2.00	2.63	9.12	2.67	30.32
W-17	D.B.	3.00	4.20	2.97	3.20	2.67	0.67	1.90	2.60	9.07	2.70	29.98
W- 6	D.B.	3.09	4.34	3.01	3.20	2.62	0.71	1.94	2.62	8.84	2.56	29.84
W- 9	D.B.	3.12	4.39	3.05	3.33	2.69	0.67	1.96	2.66	8.97	2.60	30.32
W-15	D.B.	3.19	4.01	2.88	3.20	2.67	0.69	1.88	2.57	8.81	2.57	29.28
W-14	D.B.	3.35	4.24	2.96	3.22	2.54	0.66	1.97	2.66	8.72	2.57	29.54
W- 1	L.B.	4.03	4.22	2.80	3.20	2.71	0 67	1.89	2.58	8.51	2.61	29.19
Mean		3.02	4.24	3.01	3.30	2.65	0.69	1.96	2.65	8.99	2.68	30.17
Newal barley												
B-10	G.	1.83	4.21	2.73	3.55	2.24	0.71	2.19	2.30	10.05	4.21	32.19
B-19	G.	2.00	4.50	2.85	3.65	2.55	0.75	2.35	2.35	9.70	3.90	32.60
B-21	G.T.	2.07	4.54	2.80	3.77	2.56	0.77	2.32	2.42	10.24	4.01	33.43
B-25	G.T.	2.32	4.48	2.76	3.62	2.54	0.73	2.37	2.50	9.44	3.84	32.28
B-11	Bl.T.	2.56	4.26	2.70	3.56	2.19	0.70	2.23	2.38	9.57	4.06	31.65
B- 9	D.B.	2.58	4.38	2.71	3.57	2.60	0.70	2.25	2.44	9.54	3.64	31.83
B-14	D.B.	2.81	4.48	2.71	3.59	2.71	0.71	2.21	2.53	9.04	3.63	31.61
B-20	Bl.	2.98	4.46	2.72	3.59	2.72	0.74	2.22	2.35	8.76	3.66	31.22
B- 1	L.B.	3.10	4.23	2.71	3.58	2.84	0.61	2.07	2.36	9.03	3.61	31.04
Mean		2.47	4.39	2.74	3.61	2.55	0.71	2.25	2.40	9.49	3.84	31.98
Victory oats												
O-10	G.	1.51	4.37	3.31	4.11	2.32	0.66	2.12	2.05	11.85	3.78	34.57
O-19	G.	1.67	4.43	3.29	4.19	2.34	0.72	2.16	2.16	11.80	3.77	34.86
O-24	G.	1.99	4.72	3.37	4.27	2.56	0.75	2.26	2.21	12.01	3.82	35.97
O-12	G.	2.16	4.68	3.43	4.21	2.50	0.69	2.22	2.32	12.59	3.98	36.62
O-26	G.T.	2.31	4.55	3.25	4.11	2.34	0.69	2.21	2.25	11.95	3.90	35.25
O- 7	D.B.	2.50	4.72	3.28	4.20	2.56	0.72	2.24	2.40	12.52	3.76	36.40
O- 8	D.B.	2.54	4.88	3.31	4.29	2.52	0.71	2.28	2.40	12.68	4.02	37.09
O-16	D.B.	2.71	4.95	3.36	4.50	2.77	0.66	2.21	2.29	12.40	4.10	37.24
O-13	L.B.	3.15	4.67	3.18	4.00	2.44	0.67	2.19	2.44	12.67	3.75	36.01
Mean		2.28	4.66	3.31	4.21	2.48	0.70	2.21	2.28	12.27	3.88	36.00

[1] G. = gray-wooded; G.T. = gray transitional; Bl. = black; Bl.T. = black transitional; D.B. = dark brown; L.B. = light brown.

grain protein quality as measured by the proportion of the 9 essential amino acids under consideration. In a recent paper Sheldon, Blue and Albrecht ('48) indicate that the quality of lespedeza and alfalfa protein is affected by soil zone and by fertilizer treatment, but inasmuch as the data presented do not show the total nitrogen or protein content of the material considered, it is not clear whether their experiments demonstrate marked changes in quality, or simply in quantity, of protein produced on different soils and under different treatments.

TABLE 2

Amino acid content of the protein (N × 6.25) of 9 samples of Marquis wheat, Newal barley and Victory oats. (Data are reported on a moisture-free basis)

	MARQUIS WHEAT		NEWAL BARLEY		VICTORY OATS	
	Mean	Range	Mean	Range	Mean	Range
	%	%	%	%	%	%
Leucine	6.4	6.0–6.6	6.6	6.2–6.8	7.0	6.6–7.3
Isoleucine	4.5	4.2–4.8	4.1	4.0–4.2	5.0	4.8–5.2
Valine	4.4	4.3–4.8	4.9	4.7–5.0	5.7	5.4–5.9
Phenylalanine	5.0	4.8–5.1	4.8	4.2–5.2	4.7	4.3–5.1
Methionine	1.2	1.1–1.3	1.2	1.0–1.3	1.2	1.1–1.3
Threonine	2.7	2.6–2.8	3.0	2.8–3.2	3.0	2.9–3.1
Histidine	1.6	1.5–1.7	1.4	1.4–1.5	1.3	1.2–1.5
Arginine	4.5	4.2–4.7	4.7	4.4–5.1	6.1	5.8–6.3
Lysine	2.2	2.1–2.5	3.2	3.0–3.5	3.3	3.1–3.4
Total 9 amino acids	32.5		33.9		37.3	

Species differences

In table 2 the assay results are briefly summarized in terms of per cent amino acid of total N × 6.25. From these data or those in table 1 it may be seen that in the samples under assay differences exist among the proteins of Marquis wheat, Newal barley and Victory oats with respect to concentrations of certain of the amino acids. For example, the mean lysine value determined for Newal barley and Victory oat protein is 1.4 to 1.5 times that obtained for the protein (N × 6.25) of Marquis wheat.

Fiducial limits were calculated from the assay data — a separate standard error being calculated for each class of data — to determine the probability of the existence of differences among the proteins of the three grains with respect to concentrations of lysine, arginine, valine, threonine, leucine, isoleucine and histidine. For the last three amino acids the probability of inter-species differences was less than 20:1. The comparisons which showed a probability greater than 20:1, i.e., where there was no overlapping of the respective 5% fiducial limits of the two means being compared, are presented in table 3.

TABLE 3

Probability of inter-species differences

Concentration of	PROBABILITY THAT		ODDS IN FAVOR
	In	Exceeds that in protein of	
Lysine	Victory oats	Marquis wheat	> 100:1
Lysine	Newal barley	Marquis wheat	> 20:1
Arginine	Victory oats	Marquis wheat	> 20:1
Arginine	Victory oats	Newal barley	> 20:1
Valine	Victory oats	Marquis wheat	> 20:1
Valine	Newal barley	Marquis wheat	> 20:1
Valine	Victory oats	Newal barley	> 20:1
Threonine	Victory oats	Marquis wheat	> 20:1
Threonine	Newal barley	Marquis wheat	> 20:1

These results, based on assays of 9 samples of each grain, indicate that the protein of Marquis wheat is inferior to that of either Victory oats or Newal barley with respect to content of lysine, and that it is probably inferior with respect to two other amino acids, threonine and valine, which may be required in supplemental amounts in grain rations. Although the significance of this relationship may be small in practical feeding, since all three grains are relatively poor sources of these amino acids, the possibility is nonetheless indicated that the use of protein supplements of high quality may be especially important in livestock and poultry rations containing a high proportion of wheat. In this connection it should,

however, be noted that Doty, Bergdoll, Nash and Brunson ('46) have shown that the amounts of certain amino acids in corn protein are, to some extent, affected by the genetic constitution of the plant. If future work with other cereal grains should demonstrate the existence of significant varietal differences within species, another complicating factor would have to be taken into consideration in any attempt to draw conclusions regarding the relative adequacy of the protein of different species of cereal grain.

SUMMARY

1. Results are reported of microbiological assays for 9 essential amino acids in 9 samples of one variety each of wheat, barley and oats.

2. Variability was observed in the proportion of the total nitrogen contributed by the nitrogen of a given amino acid in different samples of a variety within a species. This variability was most evident for the amino acids lysine, arginine, valine and phenylalanine.

3. The possibility is discussed that the fraction of the total nitrogen contributed by the nitrogen of lysine may decrease as the total nitrogen content of grain increases.

4. Assay values obtained with samples grown under a wide range of soil and climatic conditions failed to demonstrate the existence of a significant correlation between soil zone and grain protein quality as measured by the proportion of 9 essential amino acids in grain proteins.

5. Evidence is presented indicating that the protein of Marquis wheat may contain significantly less of certain of the essential amino acids, notably lysine, than do the proteins of Victory oats or Newal barley.

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STUDIES ON THE ADEQUACY OF HUMAN MILK AND ARTIFICIAL HUMAN MILK FOR THE RAT [1]

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ONE FIGURE

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The results of studies of the composition of human milk have shown that wide differences exist between the nutritive properties of human milk and those of cow's milk. In spite of these differences, the trend in recent years has been toward the use of cow's milk as the major ingredient in formulas for the feeding of very young infants.

It appeared desirable, therefore, to conduct further experiments on the adequacy of infant feeding formulas composed of ingredients derived largely from cow's milk. Since the opportunities to conduct such experiments on the infants themselves are obviously limited, much of the preliminary work on such investigations must, of course, be carried out with laboratory animals.

Since few studies of this character have been conducted with artificial human milks, little evidence is available as to the applicability of results obtained with an experimental animal, such as the rat, to the actual needs of an infant.

The studies presented in this report were conducted to compare, by rat feeding experiments, the nutritive properties

[1] This work was aided by a grant to Cornell University from the Western Condensing Company, San Francisco. The work was conducted in the Nutrition Laboratories of the Department of Poultry Husbandry, Cornell University.

of dehydrated human milk with those of artificial milk mixtures designed to simulate human milk. The purpose of such comparisons was not only to evaluate the respective nutritive properties of the diets for the rat, but also to provide information concerning the suitability of the rat for evaluating materials intended for infant feeding.

EXPERIMENTAL PROCEDURE

Protein analyses conducted on several samples of human milk verified the findings of others that considerable variability is encountered in protein content. Human colostrum (milk produced during the first 10 days after parturition) contains considerably more protein than mature milk (milk produced after the 10th day).

An artificial human milk was compounded in dried form by the addition of lactalbumin, lactose, and unsalted butter to dried whole cow's milk, to give a gross chemical analysis similar to that of the average human colostrum. Thus, this artificial human milk contained 13.4% protein, 28.7% fat, and 51.5% lactose. The ratio of casein to lactalbumin was calculated to be 5:7, which is the ratio present in human milk. Minerals and known vitamins were added to this artificial milk in amounts adequate to meet all known requirements of the rat.

Experiment 1

Previous work (Mitchell and Dodge, '35; Ershoff and Deuel, '44; and others) has shown that the rat cannot tolerate high levels of lactose such as are characteristic of human milk. Also, it is generally accepted that in order to meet the amino acid requirements of the rat, the diet should contain the equivalent of 18% of casein. Therefore, the first experiment was conducted to determine if an increased protein level (18%) or additional methionine (0.44% DL-methionine) would increase rat growth when these supplements were added (1) to the basal artificial human milk and (2) to similar diets in which

part of the lactose had been replaced by glucose. The composition of the diets is presented in table 1.

The experiment was conducted with 4-week-old white rats (Sprague-Dawley) of mixed sex, three male and three fe-

TABLE 1

Composition of diets for experiment 1

LOT. NO.	COMPONENTS [1]		PROTEIN	FAT	LACTOSE	GLUCOSE
		%	%	%	%	%
1	Dried whole cow's milk	100	26.4	28.69	36.60	....
2	Dried whole cow's milk	25.0	6.6	7.17	9.15	....
	Lactalbumin	8.9	6.8	0.48	0.15	....
	Lactose	42.26	....	....	42.26	....
	Butter	21.04	....	21.04	....	....
	Totals		13.4	28.69	51.56	....
3	Dried whole cow's milk	25.0	6.6	7.17	9.15	....
	Casein	2.1	1.85	....	....	....
	Lactalbumin	12.6	9.55	0.67	0.21	....
	Lactose	36.65	....	....	36.65	....
	Butter	20.85	....	20.85	....	....
	Totals		18.00	28.69	46.01	....
4	Dried whole cow's milk	25.0	6.6	7.17	9.15	....
	Lactalbumin	8.9	6.8	0.48	0.15	....
	Lactose	41.82	....	....	41.82	....
	Butter	21.04	....	21.04	....	....
	DL-methionine	0.44	0.44	....	....	....
	Totals		13.84	28.69	51.12	....
5	Dried whole cow's milk	25.0	6.6	7.17	9.15	....
	Lactalbumin	8.9	6.8	0.48	0.15	....
	Glucose	42.26	....	....	....	42.26
	Butter	21.04	....	21.04	....	....
	Totals		13.4	28.69	9.30	42.26
6	Dried whole cow's milk	25.0	6.6	7.17	9.15	....
	Casein	2.1	1.85	....	....	....
	Lactalbumin	12.6	9.55	0.67	0.21	....
	Glucose	36.65	....	....	....	36.65
	Butter	20.85	....	20.85	....	....
	Totals		18.00	28.69	9.36	36.65
7	Dried whole cow's milk	25.0	6.6	7.17	9.15	....
	Lactalbumin	8.9	6.8	0.48	0.15	....
	Glucose	41.82	....	....	....	41.82
	Butter	21.04	....	21.04	....	....
	DL-methionine	0.44	0.44	....	....	....
	Totals		13.84	28.69	9.30	41.82

[1] The following supplements were added to all experimental diets: Na_2HPO_4, 1.10%; $CaCO_3$, 0.70%; plus 1% of the following mixture: Starch, 6.9 gm; $FeSO_4 \cdot 7H_2O$, 1.2 gm; $MnSO_4 \cdot 4H_2O$, 1.44 gm; $CuSO_4 \cdot 5H_2O$, 0.078 gm; choline chloride, 1.0 gm; Ca pantothenate, 0.005 gm. Thiamine chloride in the amount of 0.001 gm was added to all diets.

male rats per lot. The rats were housed in wire cages in a thermostatically controlled room. Feed and water were supplied ad libitum. The experiment was conducted for a 4-week period. The rats were weighed weekly, and daily feed consumption was recorded. The results of the experiment are presented in table 2.

TABLE 2

Comparison of artificial human milk with whole cow's milk for rat growth (experiment 1)

LOT OR DIET NO.	TREATMENT	INITIAL WT. (4 weeks)	FINAL WT. (8 weeks)	GAIN IN WT. FOR THE 4-WK. PERIOD	EFFICIENCY OF FOOD UTILIZATION
		gm	*gm*	*gm*	*gm food/ gm gain*
1	100% whole cow's milk	70.16	182.83	112.67	2.32
	Artificial human milk containing:				
2	13.4% Protein Lactose	69.33	117.49	48.16	4.32
	Modified artificial human milks containing:				
3	18.0% Protein Lactose	71.66	116.83	45.17	4.49
4	13.4% Protein + Methionine Lactose	68.2	123.4	55.2	4.35
5	13.4% Protein Glucose	75.5	153.0	77.5	3.72
6	18.0% Protein Glucose	70.0	156.8	86.8	3.53
7	13.4% Protein + Methionine Glucose	76.2	155.5	79.3	3.64

The data show that none of the experimental diets was entirely adequate for the rats, since the positive control lot receiving whole cow's milk enriched with certain vitamins and minerals produced greater gains than did any of the other lots.

Since the whole cow's milk diet contained more lactose than diets 5, 6 and 7, the lower growth in the latter three lots of rats could not be explained on the basis of excess lactose. The whole milk diet contained 26.4% protein, while the highest protein level among the experimental lots was 18%. This might be interpreted to indicate that the former protein level was more nearly optimum for the rat than was the latter. However, 18% protein has long been accepted as being adequate for the rat. On the other hand, the whole milk diet contained 4 times as much of the unidentified vitamins present

TABLE 3

Effect upon rat growth of yeast and liver paste supplementation of modified artificial human milk (experiment 2)

LOT NO.	NO. RATS PER LOT	TREATMENT	INITIAL WT. (4 weeks)	FINAL WT. (8 weeks)	GAIN IN WT.
			gm	*gm*	*gm*
1	5	None [1]	63.6	128	64.4
2	4	2% dried brewers' yeast	58.5	142	83.5
3	4	0.5% liver paste	60.0	139	79.0
4	5	Yeast + liver paste	59.5	150.3	90.8
5	3	Whole cow's milk	65.7	149	83.3

[1] Basal diet was diet no. 6 from experiment 1, an artificial human milk modified to contain 18% protein and 36.65% glucose.

in milk as did any of the experimental diets, since the only source of these factors in the experimental diets was from the 25% whole milk used.

Accordingly, a second experiment was conducted to determine whether additional unidentified vitamins are required for optimum rat growth on a diet such as that used for lot no. 6 (see table 1).

Experiment 2

This experiment was conducted entirely with female rats, three to 5 rats per lot. Diet no. 6 from experiment 1 was fed alone and supplemented with 2% dried brewers' yeast and

TABLE 4

Rat growth on artificial human milks plus yeast as compared to that on whole cow's milk (experiment 3)

LOT NO.	TREATMENT [1]		INITIAL WT. (3 weeks)	FINAL WT. (7 weeks)	GAIN IN WT. FOR THE 4-WK. PERIOD	EFFICIENCY OF FOOD UTILIZATION
			gm	*gm*	*gm*	*gm food / gm gain*
1	100%	whole cow's milk	42.3	147.7	105.4	2.63
	Artificial human milk containing:					
2	13.4%	Protein Lactose	42.2	102.3	60.1	4.48
	Modified artificial human milks containing:					
3	18.0%	Protein Lactose	42.0	107.7	65.7	4.39
4	13.4%	Protein + Methionine Lactose	43.0	99.3	56.3	5.14
5	13.4%	Protein Glucose	42.8	128.8	86.0	3.30
6	18.0%	Protein Glucose	42.8	144.4	101.6	2.96
7	13.4%	Protein + Methionine Glucose	42.7	145.7	103.0	2.90

[1] Diets the same as those used in experiment 1 with the following supplements added to all experimental diets: Na_2HPO_4, 1.10%; $CaCO_3$, 0.70%; dried brewers' yeast, 2.0%; plus 1% of the following mixture: starch 6.9 gm; $FeSO_4 \cdot 7H_2O$, 1.2 gm; $MnSO_4 \cdot 4H_2O$, 1.44 gm; $CuSO_4 \cdot 5H_2O$, 0.078 gm; choline chloride, 1.0 gm; Ca pantothenate, 0.005 gm; thiamine chloride, 0.001 gm; folic acid, 0.0002 gm. Biotin in the amount of 0.0002 gm was added to all diets.

with 0.5% liver paste [2] alone and in combination. Dried whole cow's milk enriched with vitamins and minerals was again fed as the positive control. The experimental outline and results are presented in table 3. These results show that some nutrient present in both dried brewers' yeast and in the liver

[2] A 95% alcohol soluble liver fraction prepared by Wilson and Company, Chicago, Illinois.

paste is necessary for optimum growth in rats fed this type of diet.

Experiment 3

In view of the discovery that the artificial human milk was deficient in an unidentified factor or factors present in dried brewers' yeast and liver paste, a third experiment was conducted using the same diets and experimental plan used in experiment 1 except that 2% of dried brewers' yeast was added to all diets. This experiment was conducted with weanling white rats of mixed sex, three male and three female rats per lot. The results of the experiment are presented in table 4.

It is apparent from these results that the addition of dried brewers' yeast to the 18% protein-glucose diet (lot 6) produced a growth rate almost equivalent to that obtained with whole cow's milk. In fact, an equivalent rate of gain was obtained with the 13.4% protein-glucose lot (lot 7) when sufficient methionine was added to meet the requirements of the rat for this amino acid. On the other hand, all lots (2, 3 and 4) receiving the high level of lactose did not make normal gains even in the presence of dried brewers' yeast. This indicates that, at least during the first month after weaning, the rat cannot utilize lactose sufficiently well to maintain a normal growth rate on lactose levels much above 40%, even though the diet is adequate in all other nutrients. This fact should be kept in mind in evaluating all other experiments presented in this report.

Experiment 4

This experiment was conducted to compare the growth-promoting effects of dried mature human milk [3] with that of dried whole cow's milk. Both milks were supplemented with the vitamins and minerals added to the cow's milk in the previous experiment. Another lot received the human milk supple-

[3] Obtained from the Dispensary for Mothers' Milk, Louisville, Kentucky. The milk was shipped by air freight in the frozen state and was dried under vacuum at temperatures well below freezing.

mented with 2% yeast and 0.5% liver paste. The experiment was conducted with weanling white rats (Sprague-Dawley), three male and three female rats per lot in the group receiving cow's milk and two rats of each sex in the lots receiving human milk. The experimental conditions were the same as those used in the earlier experiments. The diets used and the experimental results are described in table 5.

The results of the experiment show that the rats receiving mature human milk failed to grow at all. The addition of 2% yeast and 0.5% liver paste to the mature human milk failed to improve it as a diet for the rats. Growth failure of the rats on the human milk diets could not have been due to inanition, since the rats on these diets ate almost as much feed in proportion to their size as the rats on the whole cow's milk diet. The feed consumption in all lots was consistent throughout the experiment. There was no evidence of loss of appetite at any time.

TABLE 5

Growth failure of rats on mature human milk alone and supplemented with yeast and liver paste (experiment 4)

TREATMENT [1]	BODY WEIGHT		WEIGHT GAIN IN 4 WKS.	FEED CONSUMED PER RAT, IN 4 WKS.
	Average initial	Average at 4 wks.		
	gm	*gm*	*gm*	*gm*
Cow's milk	46.0	168.7	122.7	263
Mature human milk	45.5	42.0	—3.5	110
Mature human milk + 2% yeast + 0.5% liver paste	44.8	43.5	—1.3	126

[1] Supplemental vitamins and minerals as described in footnote to table 4, omitting yeast, Na_2HPO_4, and $CaCO_3$.

Experiment 5

Since the casein of cow's milk is a much better source of factor S, a growth factor for chicks, than is yeast or liver paste

(Scott, Norris and Heuser, '47), it was decided to conduct an experiment to determine if factor S is required by the rat and is deficient in mature human milk. Accordingly, one lot of rats received human milk alone. Another received human milk plus 5% crude casein. A third lot received human milk plus the pure amino acids equivalent to 5% casein. Each lot contained two of the rats which had been on the human milk diets in experiment 4.

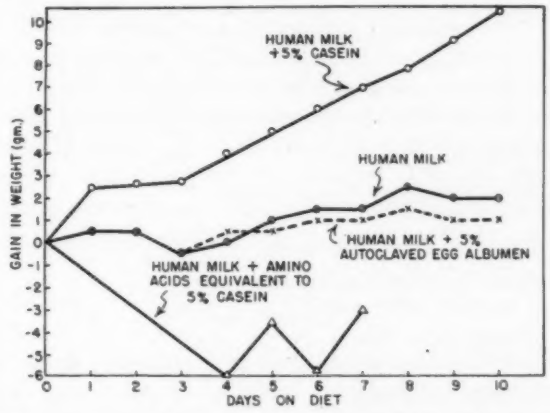

Fig. 1 Results of experiment 5 showing growth obtained when mature human milk was supplemented with crude casein, a source of factor S.

Since the human milk used in these experiments contained only 9% protein, it was considered possible that a growth response due entirely to the additional protein provided might be obtained from supplementation with 5% casein. Therefore, as a second check on this, one lot was fed human milk plus 5% autoclaved egg albumen, a relatively poor source of factor S. In this way the protein was increased to approximately the same level as prevailed for the lot receiving casein without the addition of factor S.

The results of this experiment, presented in figure 1, show that neither egg albumen nor the amino acids caused any growth, but that supplementation with casein produced a good

rate of growth. These results indicate, therefore, that the rat does require factor S and that mature human milk is deficient in this factor.

Experiment 6

This experiment was conducted to determine if human colostrum promotes growth in rats without supplementation with

TABLE 6

Results of experiment 6 showing slow growth of rats on human colostrum not improved by additional casein (factor S)

LOT NO.	TREATMENT [1]	INITIAL WT. (3 weeks)	FINAL WT. (6 weeks)	GAIN IN WT.
		gm.	gm.	gm.
1	Human milk (colostrum)	50.2	78.5	28.3
2	Human colostrum + 5% autoclaved egg albumen	51.0	71.0	20.0
3	Human colostrum + 5% casein	51.5	76.0	24.5
4	Human colostrum + amino acids equivalent to 5% casein	51.7	74.8	23.1
5	Artificial human milk (12% protein)	51.3	72.0	20.7
6	Artificial human milk (13.4% protein)	53.5	97.5	44.0
7	Whole cow's milk	50.1	125.5	75.4

[1] Supplemental vitamins and minerals as in experiment 4, table 5.

factor S. The human milk for this study [4] was collected largely from mothers who were in their first week to 10 days of lactation. This milk differed from that used in experiments 4 and 5, which was largely mature milk. The protein content of the dried colostrum was found to be 11.9%, approximately 3% higher than that in the human milk used in the earlier experiments.

Two male and two female weanling white rats were used in each lot in the experiment. One lot received the human milk

[4] Also obtained from the Dispensary for Mothers' Milk, Louisville, Kentucky.

alone. Three other lots received human milk supplemented with 5% egg albumen, 5% casein, and amino acids equivalent to 5% casein, respectively. A fifth lot received the artificial human milk in which the protein level was adjusted to 12% by omission of part of the lactalbumin. This was done to make the artificial human milk have a protein content comparable to that of human colostrum. The sixth lot received the artificial human milk containing 13.4% protein, and the seventh lot received whole cow's milk as a positive control. The results of the experiment are presented in table 6.

These results show that human colostrum promotes growth in rats which is as good as that obtained with an artificial human milk having the same protein content as human colostrum, though not as good as that obtained on the artificial human milk containing 13.4% protein. The rat growth on human colostrum was not affected by addition of casein, indicating that this material is not deficient in factor S for the rat.

DISCUSSION

Experimental results have been presented which show that mature human milk, even when supplementd with vitamins and minerals to meet the known requirements of the rat for these nutrients, is entirely unsuitable as a diet for the rat. On the other hand, human colostrum, supplemented in like manner, does support a slow rate of growth. An artificial human milk formulated to contain the same gross chemical composition as that of human colostrum promoted approximately the same growth rate. Artificial human milk containing a slightly higher level of protein promoted considerably better growth than was obtained on human colostrum; increasing the protein content of mature human milk to approximately the same level did not result in increased growth unless a source of factor S was added also.

Evidence has been presented that the chief deficiencies for the rat in the artificial human milk containing 13.4% protein are methionine and an unknown factor present in dried brew-

ers' yeast. However, even when the diet is supplemented with these substances normal rat growth is not obtained unless a part of the lactose is replaced by glucose.

The failure of rats to grow on mature human milk is probably due to: (1) insufficient protein, more particularly protein containing adequate methionine; (2) a deficiency of the unidentified factor S; and (3) a level of lactose in excess of that which can be tolerated by the rat.

Human colostrum is probably deficient in protein, more especially the amino acid methionine, and contains too much lactose for the rat, but does not appear to be deficient in factor S.

While the rat is obviously a poor experimental animal for use in attempting to evaluate a food for young infants, the results of the studies presented in this report show that, at least as far as the rat is concerned, the artificial human milk formulated to contain a gross composition similar to that of average human colostrum is as nutritious, if not more so, than the human colostrum used in these studies.

All of the animals receiving the high lactose experimental diets developed diarrhea during the first week, which usually disappeared after about 10 days. Many of these experimental animals also developed a peculiar syndrome marked by ruffled fur, alopecia, and a reddened, swollen condition about the eyes resembling the "spectacle eye" reported to be due to biotin deficiency. This was not observed in the rats receiving mature human milk, but appeared in about one-quarter of the rats receiving human colostrum and in about one-half of the rats receiving the artificial human milks. Increased supplementation of these diets with biotin and folic acid had no effect, but supplementation with yeast and liver paste reduced the incidence about 50%.

A condition resembling this following lactose feeding has been described previously by several different groups of research workers (Mitchell, '35, '36; Yudkin and Arnold, '35; Day, '36; and Ershoff and Deuel, '44), except that in the studies described by these investigators the rats also devel-

oped cataracts, while ours did not. Mitchell ('36) and Ershoff and Deuel ('44) reported a strain difference in the development of cataracts and alopecia, both in relation to time of onset and to degree of incidence. Ershoff and Deuel report that on the same purified rations containing lactose as the carbohydrate source, the U.S.C. strain of albino rats survived for a longer period of time with less severe alopecia and cataracts than did the Long-Evans strain. These workers report that young rats fed a diet in which lactose is the sole carbohydrate fail to grow properly and die in from three to 20 days. The work of Mitchell ('35b) and Handler ('47) indicates that the galactose moiety is responsible for the apparent toxic effect of lactose and produces symptoms which are approximately the same as those observed when lactose is fed. Handler stated that increasing the protein or fat content of the diet, or both, increased the rats' survival time and at sufficiently high levels afforded almost complete protection against toxicity. These workers agree that the length of survival and severity of the syndrome are correlated with the severity of diarrhea.

Using an artificial human milk similar in gross composition to those used in the studies presented in this report, Daniel and Harvey ('47) found that rats (Sprague-Dawley) grew at a fair rate, remained normal and healthy in appearance over the experimental period of 6 weeks, and showed evidence of diarrhea only during the first week. They found, on the other hand, that when the mineral content of the diet was increased the animals had diarrhea throughout the whole experimental period. Since these workers reported that, in spite of the diarrhea, their animals appeared normal and healthy, evidence is provided that the alopecia and other symptoms observed by other workers are not necessarily caused by the same condition that produces the diarrhea.

Since the artificial human milk used by Daniel and Harvey contained some dried whole cow's milk, as did ours, and since they supplied the lactose and lactalbumin by means of a nondialyzable fraction from whey, it is possible that their diet

contained an unidentified factor required by the rat on a lactose diet which was missing from the purified diets used by Ershoff and Deuel ('44) and others.

The growth results obtained by Daniel and Harvey were similar to those obtained by us when dried brewers' yeast was added to our artificial human milk basal diet, indicating that the non-dialyzable factor in whey may be the same as that present in brewers' yeast.

The presence of such a factor, together with the high fat level, in the diets used by Daniel and Harvey and by us may explain the lack of mortality observed for animals on these diets.

Further evidence in support of this is the fact that the rats receiving mature human milk (one of the diets having the highest lactose content) did not exhibit any deficiency symptoms other than failure to grow.

SUMMARY

Experimental results have been presented which show that mature human milk, even when supplemented with vitamins and minerals to meet the known requirements of the rat for these nutrients, is entirely unsuitable as a diet for the rat. On the other hand, human colostrum, supplemented in like manner, does support a slow rate of growth. An artificial human milk formulated to contain the same gross chemical composition as that of human colostrum promoted approximately the same growth rate. An artificial human milk containing a slightly higher level of protein promoted considerably better growth than was obtained on the human colostrum.

The failure of rats to grow on mature human milk is probably due to: (1) insufficient protein, more particularly protein containing adequate methionine; (2) a deficiency of the unidentified factor S; and (3) a level of lactose in excess of that which can be tolerated by the rat.

Human colostrum is probably deficient in protein, more specifically the amino acid methionine, and contains too much

lactose for the rat, but does not appear to be deficient in factor S.

While the rat is obviously a poor experimental animal for use in attempting to evaluate a food for young infants, the results presented here show that, at least as far as the rat is concerned, the artificial human milk formulated to contain a gross composition similar to that of average human colostrum is as nutriitous, if not more so, than the human colostrum used in these studies.

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SOME PROPERTIES OF AN UNIDENTIFIED GROWTH FACTOR PRESENT IN FISH PRODUCTS [1]

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Many reports in the literature have described an unidentified factor or factors present in certain animal products and required for optimum chick growth. Attempts to concentrate and isolate the factor by different investigators (Johnson et al., '42; Richardson et al., '42; Evans, Carver and Hamm, '44; Scott, Norris and Heuser, '46; Jaffee and Elvehjem, '47; McGinnis et al., '47; Nichol and associates, '47; Robblee et al., '48; and Essary and associates, '48) have disclosed some of its properties. The following properties appear to be agreed upon by more than one laboratory. It is soluble in water and in various concentrations of ethanol and methanol; insoluble in acetone; thermostabile; adsorbed by Fuller's earth and Norit A and eluted by an ammonia-ethanol mixture; and dialyzable through a cellophane membrane.

The following experiments were conducted to obtain further information concerning the properties of the unidentified factor or factors present in sardine fish meal and condensed fish solubles.

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[2] Present address: Commercial Solvents Corporation, Terre Haute, Indiana.

EXPERIMENTAL

Sardine fish meal and condensed sardine fish solubles were the basic materials for the preparations as is indicated in table 1.

Extraction with ethanol

A quantity of sardine fish meal was exhaustively extracted with ethyl ether, dried, and refluxed with 80% ethanol for 5 hours. The mixture was filtered and a fresh quantity of 80% ethanol added. This refluxing process was repeated 4 times, for a total of 20 hours of refluxing. The combined filtrates were consolidated and the ethanol removed by distillation under reduced pressure. The extract was adjusted to pH 4.5 with HCl to prevent decomposition. The extracted sardine fish meal was dried at 80°C.

Enough 95% ethanol was added rapidly to a quantity of condensed fish solubles to form an 80% ethanol solution. This solution was stirred for 30 minutes and filtered. The precipitate was resuspended in a fresh quantity of 80% ethanol, stirred for 30 minutes and filtered. The precipitate was washed with a small quantity of 80% ethanol and dried at 80°C. The filtrates were combined and the ethanol removed by distillation under reduced pressure. The remaining concentrated solution was adjusted to pH 4.5 with HCl.

Adsorption with Darco G-60

A quantity of Darco G-60, equivalent by weight to twice the solids present in the extract, was added to a water or 80% ethanol solution of the active material extracted by 80% ethanol from sardine fish meal or condensed fish solubles, adjusted to pH 3.0 with HCl. The mixture was stirred for 30 minutes and filtered. The process was repeated on the filtrate twice more with fresh amounts of Darco G-60, one-half as much the second time and one-quarter as much the third time. The non-adsorbed filtrates were consolidated and concentrated at room temperature before a fan. The Darco G-60

portions were combined and stirred for 30 minutes with an equivalent quantity by weight of 10% ammonium hydroxide in 95% ethanol. The mixture was filtered and the residue treated twice more with similar fresh quantities of the eluting mixture. The filtrates were consolidated and concentrated before a fan at room temperature.

Precipitation with 75% acetone

A water solution of the adsorbed fraction of an 80% ethanol extract of sardine fish meal was poured with stirring into 100% cold acetone to form a 75% acetone solution. The mixture was refrigerated at $-10°C.$ for two hours, filtered, and the precipitate washed with small quantities of cold acetone. The acetone was removed from the filtrate by concentrating before a fan at room temperature. The precipitate was redissolved in distilled water.

Precipitation with $(NH_4)_2SO_4$

A solution of adsorbed 80% ethanol soluble condensed fish solubles was saturated with ammonium sulfate. The mixture was stirred for one hour and filtered. Excess ammonium sulfate was removed from the filtrate and precipitate by precipitation with $Ba(OH)_2$.

Isoelectric precipitation

A solution of 80% ethanol soluble condensed fish solubles was adjusted to pH 3.0 with HCl, and filtered. The precipitate was washed with small quantities of pH 3.0 water and then redissolved in distilled water at pH 7.0.

Extraction with n-butanol

A solution of 80% ethanol soluble condensed fish solubles was extracted 4 times with equal quantities by volume of *n*-butanol. The *n*-butanol was removed by concentration before a fan at room temperature and the extracted material redissolved in pH 7.0 water.

Testing of preparations

Chick feeding experiments were conducted to test the potency of the various fractions prepared either from sardine fish meal or sardine condensed fish solubles. The experiments consisted of 9 trials with mixed sexes of single-comb White Leghorn chicks hatched from hens that had been on the same all-plant protein ration. The experimental chicks were housed in electrically heated batteries with raised screen floors and for the first two weeks after hatching all chicks were fed the basal diet, which consisted in percentages of yellow corn 43.25; soybean oil meal (mixture of 4 commercial expeller sources) 50.00; dehydrated alfalfa meal (17% protein) 3.00; dried fermentation solubles (500 μg of riboflavin per gram) 0.50; steamed bone meal 1.50; ground limestone 1.00; iodized salt 0.50; and cod liver oil (2000 U.S.P. units of vitamin A and 400 A.O.A.C. units of vitamin D per gram) 0.25. Sixty milligrams of manganese sulfate and 4.50 mg of niacin were added to each pound of this ration. Feed and tap water were supplied ad libitum. At the end of the second week the chicks were leg-banded, weighed, and selected for the experiment. All the very light and very heavy birds were discarded. Those of intermediate weight were divided into groups of 20 chicks according to individual weights, so that each group was balanced with every other group on a weight basis. The chicks were weighed individually at weekly intervals, and at the same time feed consumption was recorded. The duration of the trials was 6 weeks. All preparations were added to the basal ration. The effects of the various fractions of sardine fish meal and condensed fish solubles upon the growth of the chicks are summarized in table 1.

RESULTS AND DISCUSSION

All results are expressed as "response" rather than as average weights to facilitate a direct comparison of the results of different trials. In each trial the unsupplemented basal group and the group fed the basal ration supplemented with

TABLE 1

The comparative growth response of chicks fed fish product fractions at levels equivalent to fish product in positive control ration, or as indicated

PREPARATION	ADDED SOLIDS OF PPTN. IN RATION [1]	RESPONSE EXPRESSED AS PER CENT OF DIFFERENCE IN GROWTH BETWEEN POSITIVE AND NEGATIVE CONTROL GROUPS [2]								
		Trial								
		1	2	3	4	5	6	7	8	9
	%									
(1) Fish meal insoluble in 80% ethanol	2.217	—5	—2	..	..	..	..	..	..	..
(2) Fish meal soluble in 80% ethanol	0.180	84	66	..	..	..	..	..	..	..
(3) Fish meal soluble in 80% ethanol ≅ 6%	0.382	92	99	124	76	98	56	..	..	..
Adsorbed by Darco G-60 at pH 3 from 80% ethanol solution of (3) ≅ 6%	0.066	..	..	35	28	41	..	..	..	..
Non-adsorbed by Darco G-60 at pH 3 from 80% ethanol solution of (3) ≅ 6%	0.183	..	..	106	66	96	..	..	..	..
Non-adsorbed by Darco G-60 at pH 3 from a water solution of (3) ≅ 6%	0.119	—2	14	..	..	—1	..	..	..	..
(4) Adsorbed by Darco G-60 at pH 3 from a water solution of (3) ≅ 6%	0.132	81	92	..	..	95	89	..	..	..
75% acetone-soluble of (4) ≅ 6%	0.119	92	79	..	..	..	..	..	..	..
75% acetone-insoluble of (4) ≅ 6%	0.027	63	54	..	..	..	..	..	..	..
Adsorbed by Darco G-60 from 80% ethanol solution of (4) ≅ 6%	0.022	..	..	..	..	..	—20	..	..	..
Non-adsorbed by Darco G-60 from 80% ethanol solution of (4) ≅ 6%	0.070	..	..	..	..	..	24	..	..	..
(5) Fish solubles adsorbed by Darco G-60 at pH 3	0.199	..	..	..	..	..	..	77	61	..
(6) Fish solubles non-adsorbed by Darco G-60 at pH 3	0.330	..	..	..	..	..	..	—11	35	..
(7) Fish solubles insoluble in 80% ethanol	0.440	..	..	..	..	..	..	16	13	..
(8) Fish solubles soluble in 80% ethanol	0.417	..	..	..	..	84	..	121	94	91
Non-adsorbed by Darco G-60 from water solution of (8)	0.173	..	..	..	..	—19	..	..	..	..
(9) Adsorbed by Darco G-60 from water solution of (8)	0.168	..	..	..	..	76	..	..	..	95
Adsorbed by Darco G-60 from 80% ethanol solution of (9) ≅ 4%	0.054	..	..	..	..	..	..	..	..	12
Non-adsorbed by Darco G-60 from 80% ethanol solution of (9) ≅ 4%	0.048	..	..	..	..	..	..	..	..	62
n-butanol soluble of (9) ≅ 4%	0.036	..	..	..	..	..	..	..	..	39
n-butanol insoluble of (9) ≅ 4%	0.153	..	..	..	..	..	..	..	..	88
pH 3 soluble of (9) ≅ 4%	0.180	..	..	..	..	..	..	..	..	65
pH 3 insoluble of (9) ≅ 4%	0.020	..	..	..	..	..	..	..	..	2
Saturated ammonium sulfate-soluble of (9) ≅ 4%	0.091	..	..	..	..	..	..	..	..	13
Saturated ammonium sulfate-insoluble of (9) ≅ 4%	0.112	..	..	..	..	..	..	..	..	62

[1] PPtn = preparation.
[2] Level of fish product in positive control ration: Sardine fish meal, 3%; fish solubles, 2%.

3% sardine fish meal or 2% condensed fish solubles serve as negative and positive controls. The per cent response is calculated by considering the difference between the negative and positive controls as 100%. A response of zero indicates that the growth of chicks fed the supplemented ration was the same as that with the basal ration, while a response of 100 indicates a growth equivalent to that on the original fish product supplemented ration.

The growth factor, or factors, was extracted from both the sardine fish meal and the condensed fish solubles by 80% ethanol. The factor was soluble in 75% acetone but insoluble in *n*-butanol. Most investigators have stated that the growth factor is insoluble in acetone, whereas the growth results presented in table 1 show that the factor was soluble under the conditions of the present experiments. This is in agreement with Bird et al. ('48), who observed that the growth factor present in cow manure was soluble in 80% acetone.

The activated charcoal, Darco G-60, adsorbed the activity at pH 3.0 from a water solution of an 80% ethanol extract of sardine fish meal or condensed fish solubles. This activity could easily be eluted with 10% ammonium hydroxide in 95% ethanol, with but 20% loss of activity. McGinnis et al. ('47), on the other hand, reported that the factor was not adsorbed by activated charcoal at pH values from 2.0 to 5.0. Their results may indicate a possibility that more than one factor is involved, but a single factor could be responsible for the results noted since their experiments were carried out under different conditions and with liver preparations. In addition, in the experiments reported in this paper Darco G-60 was unable to adsorb the factor from an 80% ethanol soluton. The pigments present in the crude extracts were adsorbed, leaving the active material in a colorless filtrate.

Precipitation of inert material was obtained by adjusting the pH of the solution to 3.0. The growth-promoting substance remained in solution. Saturated ammonium sulfate precipitated the active material, but there is no definite information

concerning the association of the factor with protein in the ammonium sulfate precipitate.

The unidentified factor or factors in fish products examined in these studies exhibited "animal protein factor" activity and properties similar to those reported by other investigators. Recently Ott, Rickes and Wood ('48) reported that crystalline vitamin B_{12} elicited growth response in chicks comparable to that obtained with crude sources of the animal protein factor, indicating that vitamin B_{12} is identical with or closely related to this factor. Further work will be required to determine whether the growth factor, or factors, in fish products is identical with vitamin B_{12} or comprises a combination of this vitamin and a closely related factor.

SUMMARY

The essential chick growth factor present in sardine fish meal and condensed fish solubles was soluble in 75% acetone and in 80% ethanol. It was insoluble in *n*-butanol. The factor was adsorbed by Darco G-60 at pH 3.0 from a water solution of an alcoholic extract of sardine fish meal or condensed fish solubles, but not adsorbed from an 80% ethanol solution of the same extract. Elution of the factor from Darco G-60 can be accomplished with 10% ammonium hydroxide in 95% ethanol with about 20% loss of activity. The factor was colorless in solution.

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THE PANTOTHENIC ACID REQUIREMENTS OF CHICKS RECEIVING A PURIFIED DIET[1]

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ONE FIGURE

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Although there have been several reports relating to the subject, the pantothenic requirement of the chick is still debatable. The original figure of 1400 μg per 100 gm of diet found by Jukes ('39) was approximately twice that reported by Baurenfeind and co-workers ('42). More recently Lepkovsky et al. ('45) report that somewhere between 460 and 970 μg per 100 gm of diet are required. The same authors estimate that the turkey poult has a somewhat higher requirement, between 970 and 1170. Hegsted and Perry ('48) have estimated the requirement of ducklings to be approximately 1100 μg per 100 gm of diet.

The recent findings of Lipmann and co-workers ('47b) offer a possible explanation for the discrepancies among these studies. They have shown that much of the pantothenic acid in foods is combined in the form of the more complex compound designated coenzyme A, the pantothenic acid of which is unavailable to *L. casei* by the usual methods of microbiological assay. Hegsted and Lipmann ('48) showed that the pantothenic acid in coenzyme A is also only partially

[1] Supported in part by grants-in-aid from the Milbank Memorial Fund, New York, New York; the Nutrition Foundation, Inc., New York, New York; and Swift and Company, Chicago, Illinois.

available to the chick. The over-all average of several tests indicated a mean availability of only 66% of the total pantothenic acid in the compound. It seems likely, therefore, that the different basal diets used in the several studies on pantothenic acid requirements may have varied in pantothenic acid content as well as having had various degrees of availability. In this regard it is of interest that the difference in the results obtained by Lepkovsky et al. ('45) with two different diets may be accounted for principally by the difference in the pantothenic acid content of the diets as determined microbiologically.

We report in this paper a study of the pantothenic acid requirements of chicks fed a purified diet rather than the heated diets previously used. Since the basal diet probably contains only an insignificant amount of pantothenic acid, the results should not be complicated by the presence of combined pantothenic acid of an unknown degree of availability. Also the results should be comparable to those obtained in the previous study upon the pantothenic acid requirements of ducklings (Hegsted and Perry, '48), in which a similar diet was used.

EXPERIMENTAL

The pantothenic acid low diet was the same as that described by Hegsted and Perry ('48) except that the corn oil was reduced by 5% and the glucose increased by this amount. A total of 126 White Leghorn chicks were used. They received a standard chick mash until they were one week old and were then divided into 21 groups of 6 chicks each and given a pantothenic acid low diet for 5 days. After this partial depletion they were given the same diet to which various levels of calcium pantothenate had been added. Two groups (6 per group; 12 chicks per level) received diets containing 130, 300, and 500 μg per 100 gm of diet and three groups (6 per group; 18 chicks per level) received diets containing 700, 1000, 1500, 2500, and 4500 μg per 100 gm of diet. The chicks were weighed thrice weekly for three weeks. When they were

killed, the livers from each group were removed immediately, weighed, homogenized together in water, and the suspension heated to boiling. An aliquot was frozen and later analyzed microbiologically for total pantothenic acid, utilizing pigeon liver extract and phosphatase to free the bound acid, since this has been shown to release the total bound vitamin (Lipmann, Kaplan and Novelli, '47a).

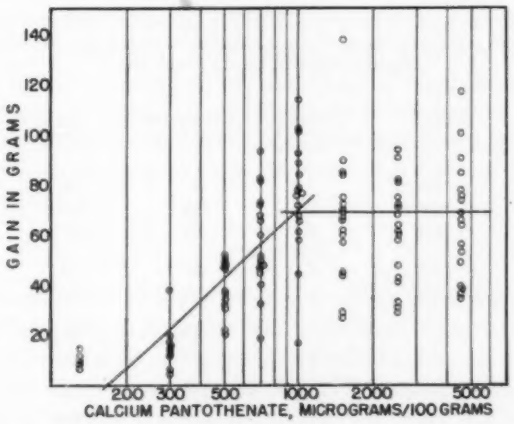

Fig. 1 Scatter diagram showing the relation between the calcium pantothenate content of the diet and the weight gain during the last 10 days of the experiment.

RESULTS

In order to minimize the effects of the previous depletion or of storage of pantothenic acid, only the gains during the last 10 days on the experiment were used. These data were analyzed as previously described (Hegsted, '48) by calculating the regression line shown in figure 1. The 130 μg level was clearly less than the maintenance requirement, since 7 of the 12 birds failed to survive, and these data were not included in calculating the line. The gains from the 1500 μg level and above were also excluded, since these appear to be above the region of linearity. The regression line bisects the abscissa

at 171 μg of calcium pantothenate per 100 gm of diet (156 μg of pantothenic acid), which is assumed to be the best estimate of the maintenance requirement. To allow a gain of 68 gm, which was the mean gain of all the chicks which received more than 1000 μg per 100 gm of diet, approximately 955 μg of calcium pantothenate (874 μg of pantothenic acid) per 100 gm of diet were required. This has been taken as the best esti-

TABLE 1

The relation between the pantothenic acid level of the diet and the liver concentration of pantothenic acid

GROUP NUMBER	DIETARY Ca PANTOTHENATE	NUMBER OF LIVERS IN SAMPLE	MEAN WEIGHT OF CHICKS [1]	MEAN WEIGHT OF LIVERS	PANTOTHENIC ACID CONCENTRATION IN LIVERS
	μg/100 gm		*gm*	*gm*	*μg/mg*
1	130	1	108	4.3	40
2	130	4	93	3.5	50
3	300	6	96	4.3	56
4	300	4	98	4.3	25
5	500	5	116	5.4	71
6	500	6	122	5.6	66
7	700	6	155	7.4	62
8	700	6	145	5.9	79
9	700	5	137	5.6	54
10	1000	6	170	6.1	71
11	1000	6	193	7.7	79
12	1000	6	177	8.0	57
13	1500	3	165	6.0	68
14	1500	3	198	6.9	77
15	1500	3	148	5.7	81
16	2500	3	180	5.9	66
17	2500	3	154	5.3	74
18	2500	3	190	7.8	54
19	4500	3	167	5.3	73
20	4500	3	148	5.2	55
21	4500	3	200	7.0	80

[1] Only three chicks from each group were taken from groups 13–21. The weight shown for these groups is that of the chicks whose livers were analyzed rather than the group mean.

mate of the mean pantothenic acid requirement for growth under these conditions. It is obvious from the wide scatter of the data that the estimate is not particularly reliable.

The pantothenic acid content of the livers was of considerable interest. As may be seen from table 1, the concentration of pantothenic acid was less than normal only in the groups which received 130 and 300 μg of calcium pantothenate per 100 gm of diet. The next higher levels allowed apparently normal liver concentrations, although growth was depressed. It therefore appears that liver concentration is not a sensitive criterion of the adequacy of the diet, at least much less so than growth. A calculation of the total liver content of pantothenic acid showed a much better correlation with growth, but this was primarily a matter of liver size rather than liver concentration.

DISCUSSION

As indicated in the introduction to the present paper, one of the possible explanations for the variations in the estimates of the pantothenic acid requirement of chicks may lie in the fact that heated diets have been used which may contain unknown amounts of pantothenic acid and which may be utilized to an unknown extent. The present estimate of 900 μg per 100 gm of diet is, however, in the same range as the higher estimate made by Lepkovsky et al. ('45). It is somewhat, but probably not significantly, less than the previous figure found for the duckling using the same diet, which was 1100 μg per 100 gm of diet. As suggested by Lepkovsky and co-workers, the rate of growth may be related to the pantothenic acid needs and this may account for the higher figures obtained for ducklings and turkey poults.

The failure of the concentration of pantothenic acid in the liver to be directly related to the pantothenic acid fed, and the finding that it was a less sensitive measure of the adequacy of the diet than was the rate of growth, was somewhat unexpected since tissue analyses for several of the vitamins are thought to be rather precise measures of dietary deficiency.

In applying to practical rations estimates of requirements made with purified diets, it is clear that some consideration must be given to the possibility of the occurrence of unavailable pantothenic acid in foodstuffs. Since much of the pantothenic acid in foods is combined as coenzyme A (Lipmann, Kaplan and Novelli, '47a), and since this material itself is only partially available to chicks (Hegsted and Lipmann, '48), it may be presumed that the pantothenic acid in foods is only partially available. However, the type of material, the degree of autolysis, and so forth, would be expected to influence the amount available, and since no direct studies of the problem have been made it seems useless to speculate on whether this is relatively important or not. It does appear permissible at this time to conclude that the estimate of pantothenic acid requirement made with a purified diet is a near minimum, and that under practical conditions the requirement will be somewhat higher.

SUMMARY

The minimum requirement of pantothenic acid for maximum growth of young chicks receiving a purified diet was estimated to be approximately 900 μg per 100 gm of diet.

Analyses of the livers for pantothenic acid showed that liver concentration of this vitamin was a less sensitive criterion of deficiency than was growth.

Since it is likely that foods contain varying amounts of unavailable pantothenic acid, the value obtained with a purified diet is considered as a near minimum estimate of the requirement.

ACKNOWLEDGMENTS

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THE BIOLOGICAL AVAILABILITY OF THE CALCIUM IN BONE

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The recommended dietary allowances for calcium range from 1 gm per day for infants to 1.3–1.4 gm per day for adolescents. The allowance for adults has been set at 1 gm per day. This is increased to 1.5 gm per day during the latter part of pregnancy and 2 gm during lactation (Food and Nutrition Board, N.R.C., '48).

Present human dietaries are largely dependent on milk and milk products to supply the bulk of this calcium. This, however, was obviously not the case centuries ago, as man in the past, in common with other carnivora, was dependent on animal and fish bones for a large portion of his calcium intake. Today many aboriginal people still depend on bone as their chief source of calcium. Bone also supplies many other minerals essential for normal nutrition.

In many areas of the world the supply of milk and milk products is not sufficient to provide the recommended dietary allowances for calcium. During and immediately after the last war a large proportion of the canned meat products prepared in Canada for U.N.R.R.A. contained 15% of cooked ground bone;[1] this resulted in the final canned meat containing approximately 0.8 gm of calcium per 100 gm. Today

[1] "Cooked ground bone" was prepared commercially by cooking whole fresh bones under steam pressure until soft and then grinding the product.

all flour sold in Newfoundland, where the consumption of milk is small, contains ½ of 1% bone meal.[2] For many years bone meal has been an ingredient of certain infant cereal products.[3] Because of this use of bone, the question of the availability for humans of the calcium from this source has assumed considerable importance. It was for this reason that the present study of the comparative availability of the calcium in bone and in milk was undertaken.

EXPERIMENTAL

The experiment was carried out according to the following plan. The entire study was divided into 4 successive collection periods of 5 days each. Six subjects (4 women and two men) undertook the experimental regime. The ages of the women were 20, 24, 29 and 32, while the men were 38 and 56 years old. The experiment took place in April, so there was no excessive loss of calcium in perspiration. All subjects ate a low calcium diet devised and prepared by the Hospital for Sick Children diet kitchen and served in the staff dining-room. All portions were carefully weighed and were completely consumed by the subjects. A 7th identical portion of each item was prepared and reserved for calcium analysis. The sample meals were pooled for each collection period and the amount of calcium in the pooled sample determined. All water used in cooking and for drinking was distilled, as Toronto city water contains an appreciable amount of calcium. The menu for breakfast was unchanged throughout the study and the calcium supplement was taken at this meal. Breakfast consisted of fruit juice, cream of wheat porridge with corn syrup, white toast, butter, honey and coffee. A capsule supplying 5000 units of vitamin A and 700 units of vitamin D was taken at this meal. The menu of the other meals was varied from day to day.

[2] "Bone meal" refers to the fine dry powder obtained by boiling the bones to remove fat and a good deal of organic material, drying with hot air, and grinding.

[3] Pablum, Pabena.

The calcium supplements consisted of 50 gm of dried skim milk per subject per day (reconstituted with 150 ml of water) or 2.15 gm of bone meal. In either case the calcium supplied by the supplement was 0.646 gm per person per day. The basal diet contributed an average of 0.218 gm of calcium per day, making a total intake of 0.864 gm per day. The subjects were divided into two groups of three each. The milk or bone meal supplements were alternated between the groups and were changed for successive collection periods. That is, group 1 received a bone meal supplement for the first and third collection period and milk for the second and 4th period, while group 2 received a milk supplement for the first and third period and bone meal for the second and 4th. By planning the experiment in this way a measure of compensation was obtained for variations in the mean calcium balance that might be caused by changes in the basal menu or in the basal calcium intake. Carmine was used as a feces marker. No preliminary adjustment period was considered necessary as the basal diet plus supplement supplied a level of calcium to which the majority of the subjects was normally accustomed.

RESULTS

The experimental results are recorded in table 1. The mean balance when the calcium supplement was supplied as milk was —47 mg per day. The balance was —73 mg per day when the calcium supplement was supplied as bone meal. This loss in balance of 26 mg per day would seem to indicate a somewhat decreased availability of the calcium in bone meal compared to that in milk. However, in view of the variations in the individual results, little signficance can be attached to this observation. Statistical analysis showed this to be true, the standard error of the difference between means proving to be $\pm$ 34 mg.

A disturbing feature about these results is the negative balances observed at a calcium intake level that is usually considered adequate to maintain calcium equilibrium. Steg-

gerda and Mitchell ('46) give an average daily requirement compiled from the literature of 653 mg. In the same report a mean balance of —125 mg per day for a mean low calcium intake of 222 mg per day was reported. This was derived

TABLE 1

Calcium balances with milk or bone meal

COLLECTION PERIOD	SUPPLEMENT TO LOW Ca DIET	Ca IN BASAL DIET	TOTAL Ca INTAKE PER DAY	Ca IN URINE PER DAY	Ca IN FECES PER DAY	BALANCE
		mg	*mg*	*mg*	*mg*	*mg*
	Subject M.W.					
3	Bone meal	235	881	131	693	+ 57
4	Milk	206	852	153	703	— 4
	Subject N.J.					
1	Bone meal	215	861	158	710	— 6
2	Milk	216	863	165	648	+ 50
3	Bone meal	235	881	168	752	— 39
4	Milk	206	852	170	652	+ 30
	Subject D.D.					
1	Bone meal	215	861	132	790	— 60
2	Milk	216	863	152	628	+ 83
3	Bone meal	235	881	129	1036	—284
4	Milk	206	852	177	768	— 92
	Subject R.P.					
1	Milk	215	861	152	630	+ 79
2	Bone meal	216	863	120	792	— 49
3	Milk	235	881	137	628	+116
4	Bone meal	206	852	107	507	+238
	Subject S.J.					
1	Milk	215	861	159	800	— 97
2	Bone meal	216	863	146	778	— 61
3	Milk	235	881	159	815	— 93
4	Bone meal	206	852	127	792	— 67
	Subject T.D.					
1	Milk	215	861	178	860	—177
2	Bone meal	216	863	195	825	—157
3	Milk	235	881	194	1105	—418
4	Bone meal	206	852	222	960	—330

Mean balance for milk supplement = — 47 mg.
Mean balance for bone meal supplement = — 73 mg.
Difference of means = — 26 mg.

from data obtained from 19 subjects observed for a total of 920 man days. If this balance is assumed to apply to the present group, the utilization of the calcium in the milk supplement is only 12.1%, while that of the bone meal is 8.75%. Steggerda and Mitchell ('46) report utilizations for the calcium of milk of the order of 30% when the total calcium intake averaged 545 mg per day. While the higher total calcium intake (864 mg) in the present case would result in a decreased percentage utilization, it would not be expected to depress it to this extent.

Subject T.D. was in consistent gross negative balance throughout the experiment. This subject was accustomed to a high calcium intake in his normal diet. Furthermore, the feces were diarrheal in nature, being loose and unformed for the whole period. These two features may well account for the negative balances in this case. If the results for this subject are disregarded, the mean balance for the milk supplement becomes + 8 mg per day, and the percentage utilization becomes 20.7%. These values are both in the expected range. The balance with the bone meal supplement is then — 30 mg per day and the utilization is 14.8%.

If we separate the individual subjects into two groups, first, those who exhibited a better calcium balance when receiving bone meal, and, secondly, those who exhibited a better calcium balance when receiving milk, we find that subjects M.W., S.J. and T.D. all showed better utilization of the calcium in bone meal, while subjects N.J. and D.D. utilized the calcium of milk better than that of bone meal. Subject R.P. does not fall into either group, as the mean balance of + 98 for the two periods with milk is almost identical with the mean balance of + 95 with bone meal. By this method of comparison the availability of the calcium in bone meal would appear to be as good as that of milk. In any case, an accurate figure for the relative availability of the calcium in bone meal and in milk cannot be obtained until such time as the results of sufficient studies of this nature to warrant a statistical analysis have been published. This study indicates only that

the availability of the calcium in bone meal is comparable to that of milk.

The utilization of calcium by the rat

A variety of cooked ground bones had been prepared by Canada Packers, Limited, at the request of the authors. Experiments were set up to determine the retention of calcium from these bones by the rat. A sample of the bone meal used in the human study was included. Whole dried milk powder and calcium carbonate were used as controls. Six rats, about 6 weeks old and 50 gm in weight, were placed in each of 6 groups. The groups were matched for litter and sex. Each group was placed on a low calcium diet to which was added 0.200% calcium in the form of the substance to be tested. The Chant Robertson low mineral diet (Robertson and Doyle, '35) was used with the addition of 1.8% of Osborne and Mendel's salt mixture without the calcium salts. The diets were fed ad libitum for a period of 4 weeks, records being kept of the amount of food consumed. The rats were then killed, the gastrointestinal tracts removed, and the carcasses autoclaved in 3% acetic acid at 15 pounds steam pressure for two hours. This so softened the bones and teeth that they were readily homogenized in a Waring Blendor. The carcasses from each group were pooled and thoroughly homogenized and a sample removed for calcium analysis (Tisdall and Drake, '38).

The results are recorded in table 2. The first column lists the substances supplying the calcium supplement. The second and third columns give the total amount of calcium consumed and that supplied by the supplement only. The next two columns give the total calcium and phosphorus found in the rat carcass at the end of the feeding period. The next column illustrates that, within narrow limits, the phosphorus retention is proportional to the calcium retained. The 7th column gives the extra calcium retained over and above that in the low-calcium group. The percentage retention in the last column is calculated from the data in columns 3 and 7.

TABLE 2

The retention of calcium by rats

	CALCIUM SUPPLEMENT	TOTAL Ca CONSUMED	Ca SUPPLIED BY SUPPLEMENT	AVERAGE Ca IN RATS [1]	AVERAGE P IN RATS [1]	RATIO Ca/P	ADDITIONAL Ca RETAINED BY SUPPLEMENTED ANIMALS	SUPPLEMENTARY Ca RETAINED
		mg	*mg*	*mg*	*mg*		*mg*	%
Run no. 1	Whole milk	557	526	934	689	1.36	483	91.9
	Hard pork and beef bones	547	516	891	635	1.43	441	85.4
	Soft pork and beef bones	556	525	872	650	1.34	421	80.2
	Straight run bones	555	524	936	706	1.33	485	92.6
	$CaCO_3$	599	568	908	687	1.32	457	80.5
	Low Ca diet	31	...	451	408	1.10	...	...
Run no. 2	Low Ca diet	30	...	488	453	1.08	...	...
	Whole milk	631	601	1054	856	1.23	566	94.2
	Lunch meat + 12% bone	733	703	1086	832	1.31	598	85.1
	Dried bone meal	641	611	996	783	1.27	508	83.2
	Cooked bone (U.N.R.R.A.)[2]	613	583	915	750	1.22	427	73.3
	$CaCO_3$	642	607	1032	744	1.39	477	78.6

[1] Six rats in each group.

[2] Prepared commercially by cooking whole fresh bones under steam pressure until soft and then grinding the product. This material was used in meat products prepared in Canada for U.N.R.R.A., as mentioned in the text.

There appears to be no great difference in the retention of calcium from any of the bone products. The mean retention for all bone products was 83.3%, compared to an average retention of calcium from whole dried milk of 93%. Thus the retention of the calcium of bone is about 90% of that of milk for young growing rats. This is in the same range as the utilization found in the human study and tends to corroborate that result.

SUMMARY

A comparison has been made of the utilization by humans of the calcium of skim milk powder and of bone meal.

Six subjects received a low calcium diet for four 5-day periods. Supplements of skim milk powder or bone meal supplying the same amounts of additional calcium were given during alternate collection periods.

The availability of the calcium in bone meal appeared to be in the same range as that of milk.

A study of the retention by young rats of calcium from whole cooked ground bone is also reported.

The retention of calcium from this source was approximately 90% of the retention from whole dried milk.

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THE SULFUR AMINO ACID REQUIREMENTS OF TURKEY POULTS

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TWO FIGURES

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The importance of methionine and cystine in rations for growing chicks has been investigated extensively (Almquist, '47), but the need for these amino acids by growing poults has not been reported on. By employing an isolated soybean protein as the principal source of amino acids it was possible to devise a basal ration in which the normal growth of poults was limited by methionine and cystine deficiencies. By varying the cystine and methionine additions to the basal ration it was possible to determine the amounts of these amino acids required for optimum growth.

EXPERIMENTAL

The basal ration contained, per 100 gm: isolated soybean protein,[1] 28.0 gm; calcium gluconate, 5.0 gm; cellulose,[2] 5.0 gm; crude soybean oil, 3.0 gm; salt mixture, 2.5 gm; vitamin mixture, 2.0 gm; tricalcium phosphate, 2.0 gm; condensed fish solubles,[3] 2.0 gm; dicalcium phosphate, 1.5 gm; fish oil (1000 A, 400 D), 0.5 gm; choline chloride, 0.3 gm; cholic acid, 0.1 gm; inositol, 0.1 gm; and alpha-tocopherol acetate, 2 mg. The bal-

[1] "Alpha protein" from the Glidden Company.

[2] "Cellu flour."

[3] Kindly donated by Valley Dehydrating Company, Kingburg, California, through the courtesy of Mr. E. B. Fischel.

ance of the ration was supplied by cornstarch. The mineral mixture supplied the following in grams per 100 gm of ration: sodium chloride, 1.0; dipotassium phosphate, 0.5; magnesium sulfate, 0.3; potassium chloride, 0.3; sodium silicate, 0.2; manganese sulfate, 0.03; aluminum sulfate, 0.025; ferric oxide, 0.02; copper sulfate, 0.005; zinc sulphate, 0.005; cobalt acetate, 0.002; and potassium iodide, 0.001 gm. The vitamin mixture supplied the following in milligrams per 100 gm of ration: riboflavin, 1.0; thiamine hydrochloride, 1.0; pyridoxine hydrochloride, 1.0; calcium *(d)* pantothenate, 3.0; nicotinic acid, 10.0; pteroylglutamic acid,[4] 0.5; 2-methyl-1,4-naphthoquinone, 1.0; and biotin,[5] 0.02.

Bronze poults were fed a practical starting ration for from 6 to 12 days after hatching. They were then distributed into groups of equivalent weight and were fed the experimental rations. There were from 5 to 10 poults per group in experiments in which the levels of methionine were varied, and 9 or 10 per group in experiments in which the levels of cystine were varied. The poults were housed in electrically heated batteries with raised wire floors. The feed and water were supplied ad libitum. The experiments were continued from 9 to 14 days and the birds were weighed and examined at frequent intervals.

The isolated soybean protein was found to contain 82.6% crude protein, 1.30% methionine and 0.17% cystine.[6] The methionine content was determined by the method of McCarthy and Sullivan ('41) as modified by Grau and Almquist ('45), and cystine was measured by the method of Rossouw and Wilken-Jorden ('35) as modified by Sullivan and Hess ('37). The condensed fish solubles contained 30% crude protein and were assumed to supply to the ration 0.009%

[4] Kindly donated by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, through the courtesy of Dr. T. H. Jukes.

[5] Kindly donated by Merck and Company, Rahway, New Jersey, through the courtesy of Dr. D. F. Green.

[6] We are indebted to Miss M. Kamei of the Division of Poultry Husbandry, University of California, Berkeley, for making this determination.

methionine and 0.003% cystine, according to the values given by Lassen and Bacon ('46). By calculation on the basis of the above values, the basal ration contained 23.7% crude protein, 0.37% methionine and 0.05% cystine.

The DL-methionine [7] and L-cystine used in these studies were commercial preparations.

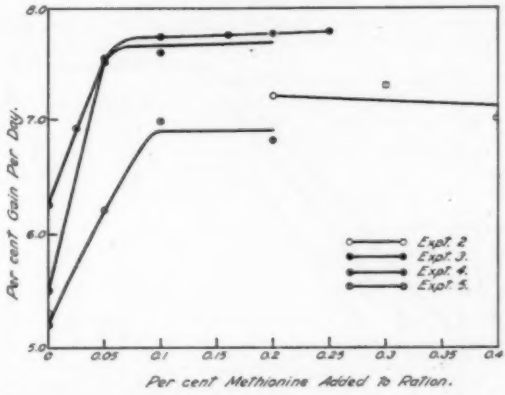

Fig. 1 The relation of the growth of turkey poults to the level of methionine added to a ration containing adequate cystine (0.55%). Notice that with levels of methionine greater than 0.1% there was no further increase in growth. The addition of cystine alone to the basal diet produced gains of only 5.2% to 6.2%.

RESULTS

When the basal ration was fed without the addition of either cystine or methionine, the gain of poults was only 0.8% per day,[8] compared with 7.0% for poults fed the same ration adequately supplemented with the sulfur amino acids. The addition of cystine to the ration produced a gain of 5.2% per day, indicating that cystine alone does not produce optimum growth.

In order to insure an excess of cystine in the ration, 0.5% cystine was arbitrarily added to the basal ration in the next

[7] See footnote 5, p. 378.

[8] Per cent gain per day $= \frac{\text{Average gain} \times 100}{\text{Average weight during experiment} \times \text{no. of days}}$.

series of trials (experiments 2–5). Various levels of methionine were added to this ration to determine the amount of methionine needed in the presence of adequate cystine. The results are shown in figure 1. Growth increased as the amount of methionine in the ration was increased until a level of between 0.05 and 0.10% methionine was reached. No significant increase was noted above 0.10%.

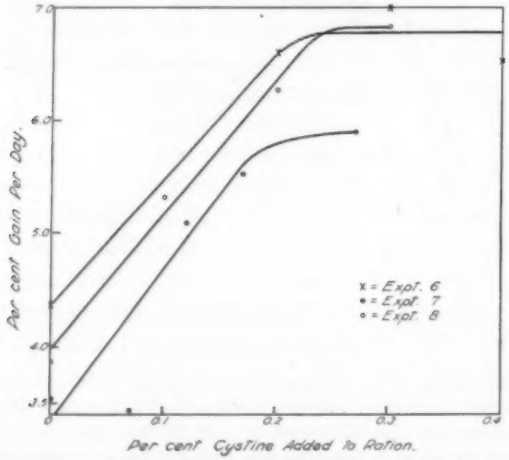

Fig. 2 The relation of the growth of turkey poults to the level of cystine added to a ration containing barely adequate methionine (0.47%). Notice that with a cystine level of about 0.25% there was no further increase in growth.

In another series of tests (experiments 6–8), 0.1% methionine was added to the ration and the level of cystine was varied. In this manner the minimum requirement for methionine was satisfied and cystine was the only factor which limited growth. The results are shown in figure 2. Growth increased as the cystine in the ration was increased until a level of approxmately 0.25% was reached.

Levels of methionine above the minimum level required for growth in the presence of cystine were included in two experiments to test the supposition that methionine can replace cystine in the ration. The pertinent data from these trials

are summarized in table 1. In the first experiment the ration containing 0.4% methionine supported growth as well as the one with adequate amounts of both cystine and methionine. In trial B, 0.3% of methionine was as effective as 0.1% methionine and 0.2% cystine.

TABLE 1

Gains of poults fed rations containing varying amounts of cystine and methionine

SUPPLEMENTS	LEVEL	MILLIMOLES S ADDED PER 100 GM RATION THROUGH			GAIN PER DAY [1]
		Cystine	Methionine	Total	
	%				%
Trial A					
Cystine	0.5	4.15	...	4.15	5.0
Cystine Methionine	0.5 0.25	4.15	1.68	5.83	6.8
Methionine	0.4	...	2.68	2.68	6.7
Trial B					
Methionine	0.1	...	0.67	0.67	3.9
Methionine Cystine	0.1 0.2	1.66	0.67	2.33	6.3
Methionine Cystine	0.1 0.3	2.50	0.67	3.17	6.8
Methionine	0.3	...	2.01	2.01	6.5

[1] For definition of "gain per day in %" see text.

Poults which were fed the purified ration with adequate supplements of the sulfur amino acids grew as well as poults fed a practical poult starting ration. In 7 experiments in which these comparisons were made, poults fed the practical starting ration grew better three times, poults fed the purified ration did better in two cases, and in two trials there was no difference in growth between birds on the two types of rations.

DISCUSSION

It has been demonstrated that in the chick methionine can be used for at least three distinct functions, namely (1) to meet the requirement for methionine itself, (2) to serve as a precursor of cystine, and (3) to serve as a methylating agent (Almquist and Grau, '45; Almquist, '47; McKittrick, '47). In the present study adequate choline was supplied in all the rations (Evans, '43) to prevent the use of methionine as a methylating agent in choline synthesis.

When 0.5% cystine was added to the ration without additional methionine, suboptimum growth resulted. Optimum growth was obtained with as low as 0.1% methionine and 0.25% cystine. This indicates that in the turkey poult an excess of cystine with adequate choline may not substitute for methionine.

Methionine may be used as a complete source of the sulfur amino acids, in contrast to cystine which will not permit maximum growth when it is the sole supplement. This indicates that in the poult, as in chicks and other animals, methionine is converted to cystine.

The amounts of methionine and cystine required to supplement the basal ration, 0.1% and 0.25% respectively, added to the quantities contained in the basal ration, 0.37% and 0.05% respectively, would place the total amounts required by the poult at approximately 0.5% methionine and 0.3% cystine. This may also be expressed as approximately 0.8% total sulfur amino acids, of which 0.5% must be supplied as methionine.

The sulfur amino acid requirement of the poult, 0.8%, is slightly less than the level of 0.9% which has been reported by Almquist ('47) to be required by the White Leghorn chick. McGinnis and Evans ('47) have reported somewhat lower values for New Hampshire chicks. Comparison of the requirements for poults and chicks is of interest since it has been found that lysine and arginine are required by poults in greater amounts than by chicks (Grau et al., '46; Kratzer

et al., '47). A deficiency of glycine was found to be less critical in the poult than in the chick (Kratzer and Williams, '48).

SUMMARY

Young turkey poults were fed rations containing an isolated soybean protein to which various additions of cystine and methionine were made. Approximately 0.5% methionine and 0.3% cystine were required for the optimum growth of the poults in a ration containing 24% crude protein. Methionine may completely replace cystine in the ration but cystine is not changed to methionine.

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COMPOSITION OF PERCHERON MARES' COLOSTRUM [1]

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It is quite generally recognized that colostrum, secreted during the first days after parturition, differs in many respects from milk produced during the remainder of the lactation period. Of late considerable attention has been devoted to the vitamin content of colostrum. Dan ('33); Henry, Houston and Kon ('40); Luecke, Duncan, Ely, Greene and Tull ('47); Spielman, Thomas, Loosli, Whiting, Norton and Turk ('47); and others have conducted studies of the carotene and vitamin content of colostrum, and Pearson, Darnell and Weir ('46) have determined the amounts of thiamine, riboflavin, nicotinic acid and pantothenic acid present in cow and ewe colostrum. Parrish, Wise and Hughes ('47) concluded that most of the fat-soluble yellow pigment in colostrum was carotene and that practically all of the vitamin A in colostrum was in the ester form. Thomas, Loosli and William ('47), and Spielman, Thomas, Loosli, Whiting, Norton and Turk ('47) established a relationship between the prepartum diet and the amount of carotene and vitamin A in bovine colostrum.

It has been recognized for some time that colostrum is far richer in protein than normal milk. More recently Smith ('46) in his studies of the immune proteins of bovine colostrum has called attention to the important role of colostrum in the transmission of antibodies from mother to offspring, and partic-

[1] Contribution No. 690, Massachusetts Agricultural Experiment Station.

ularly in the protection of the newborn calf against infectious diseases. Also Lewis and Wells ('22) concluded that there is no equivalent substitute for human colostrum for newborn infants and that it furnishes protective antibodies which add much to the newborn infant's capacity to resist infection in early life. But the literature consulted contained very little data regarding the mineral content of colostrum. Luecke, Duncan, Ely, Greene and Tull ('47) found little difference in the iron, copper, and cobalt content of the colostrum of Jersey, Holstein, and beef cows. Garrett and Overman ('40) examined the colostrum produced by purebred Holstein and Ayrshire first-calf heifers and reported that calcium, magnesium, sodium, phosphorus, and chlorine are all high at parturition and during the early hours of lactation and that they decline rather rapidly to a fairly constant level, but that potassium is rather low at parturition and gradually increases to a fairly constant level as the milk progresses toward normality.

Even less information is available regarding the mineral content of mares' colostrum, and this study was undertaken to accumulate some data regarding the mineral content and the protein and reduced ascorbic acid values of colostrum produced by purebred Percheron mares maintained under controlled conditions.

EXPERIMENTAL

The colostrum was obtained from 4 normal, well-developed, healthy Percheron mares. With the exception of mares 2a and 2b, all of the mares were used throughout the larger portion of the year as draft animals on the University farm. The ages, weights and lactation histories of the mares are as follows: Mare 1, 4 years old, 1750 lb., first lactation; mare 2a, 8 years old, 1900 lb., 5th lactation; mare 2b, 10 years old, 1900 lb., 6th lactation; mare 3, 11 years old, 1600 lb., 4th lactation; mare 4, 11 years old, 1800 lb., 5th lactation. Mares 2a and 2b are the same animal.

Colostrum samples were collected at 24-hour intervals, about 8:00 A.M., for the first 6 days of lactation. The initial samples

were obtained from mare 1, before the foal nursed; from mare 2a, about three hours after the foal was born; from mare 2b, early in the morning (the foal arrived at 10:30 P.M.); from mare 3, about 10 hours after foaling; and from mare 4, within an hour after parturition.

The study was conducted during late March and April and early May, and the mares were all confined indoors and fed home-grown, early cut mixed hay ad libitum and three quarts of crushed oats and 5 large ears of dry white dent corn per day. None of the mares had worked for several weeks preceding parturition. The mares and their foals were housed in well-lighted, adequately ventilated, earth floored, box stalls that were approximately 15 ft. square. The mares were well cared for during pregnancy, were disease-free and in excellent physical condition. Hence, the colostrum should have possessed optimum nutritive value for the breed and conditions under which it was produced.

The samples of colostrum were assayed by the recognized methods reported by the Association of Official Agricultural Chemists ('45) for water, protein, reduced ascorbic acid, phosphorus, potassium, and magnesium.

RESULTS AND DISCUSSION

The values obtained from the different assays of colostrum samples for the various constituents are reported in table 1. The amount of water in the colostrum produced by the different mares was fairly constant from day to day, and the average values, 87.5%, 86.3%, 86.3%, 88.1% and 86.3%, indicate considerable uniformity for colostrums from Percheron mares maintained under the conditions of this study. It is interesting to note that identical values were obtained for mares 2a and 2b. As stated above, the designations 2a and 2b are for different lactations of the same animal.

The protein content of the initial sample of colostrum from three of the mares was decidedly higher than for the following days, but the initial sample of colostrum from mare 1 showed less difference in protein content from the subse-

TABLE 1

Composition of Percheron mares' colostrum

MARE NO.	SAMPLE NO.[1]	WATER	PROTEIN[2]	ASCORBIC ACID	PHOSPHORUS	POTASSIUM	MAGNESIUM
		%	%	*mg/l*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*
1	1	85.2	7.7	30	58	74	24
	2	88.0	3.1	52	87	121	12
	3	87.9	..	80	99	111	14
	4	87.6	3.3	73	87	118	16
	5	88.2	3.2	84	94	86	15
	6	88.6	3.1	79	88	98	14
	Av.	87.5	4.1	66	86	101	16
2a	7	81.0	14.4	33	17	19	11
	8	86.8	3.9	64	78	83	10
	9	87.6	3.3	56	89	98	14
	10	87.3	3.6	55	90	107	14
	11	87.9	3.3	57	87	85	13
	12	87.2	3.6	75	90	87	14
	Av.	86.3	5.4	57	75	80	13
2b	13	82.5	12.1	20	44	27	19
	14	86.7	3.8	45	..	..	..
	15	87.0	3.6	91	86	95	16
	16	86.6	3.8	61	93	94	14
	17	87.4	3.4	58	88	96	16
	18	87.6	3.2	52	86	86	14
	Av.	86.3	5.0	55	79	80	16
3	19	87.7	4.0	47	80	112	18
	20	88.2	4.1	58	97	109	15
	21	87.2	3.8	53	88	93	15
	22	88.2	3.8	57	92	108	16
	23	88.4	3.3	60	72	81	14
	24	88.6	3.4	62	83	94	16
	Av.	88.1	3.7	56	85	100	16
4	25	80.1	14.2	32	46	85	26
	26	87.4	3.9	33	110	116	14
	27	86.9	3.6	44	88	94	14
	28	87.4	3.1	61	73	85	10
	29	87.8	3.3	54	94	94	12
	30	88.1	3.1	56	89	92	13
	Av.	86.3	5.2	47	83	94	15

[1] Colostrum samples were collected at 24-hour intervals, about 8: 00 A.M., for 6 consecutive days.

[2] Protein = nitrogen × 6.38.

quent samples. Although means of values that vary widely are likely to be of limited service, it may be noted that the mean amount of colostral protein in the 6 samples of colostrum from mare 2a was 5.4% and from mare 2b was 5.0%. Excluding the initial samples, the protein values were very uniform and the mean values were 3.54% for mare 2a and 3.56% for mare 2b, i.e., in successive lactation periods for the same animal. These values are definitely higher than the 2.7%, 2.4%, 2.1%, and 2.0% reported by Holmes, Spelman, Smith and Kuzmeski ('47) for Percheron mares' milk produced between the 12th and 129th days of lactation.

The amount of reduced ascorbic acid in the colostrum of each mare increased during the experimental period. The values for the amounts of ascorbic acid in the 6 samples, as seen in table 1, averaged 66, 57, 55, 56, and 47 mg/l, for mares 1 to 4, respectively. Again the values for mare 2a and 2b are in close agreement. For comparison it may be noted that milk from mares 1, 2 and 4, at a later stage of lactation, was assayed for reduced ascorbic acid. During a 5-week period, 25 samples of milk collected from each mare averaged as follows in milligrams per liter: Mare 1, from the 126th to the 162nd day of lactation, 171; mare 2b, from the 125th to the 161st day of lactation, 146; mare 4, from the 94th to the 130th day of lactation, 134. The higher values for ascorbic acid during the later stages of lactation doubtless were affected by the change in the mares' ration from dry hay and grain to unlimited amounts of grass in a good fresh pasture.

The amount of phosphorus in the initial samples of colostrum was smaller than for the succeeding samples. After the first day the phosphorus content of the colostrum was fairly constant for the individual mares. The average phosphorus contents in mg/l for the 6-day periods were 86, 75, 79, 85 and 83 for mares 1 to 4, inclusive. The values 75 mg and 79 mg per liter were for successive lactations of the same mare (2a and 2b). These values for the phosphorus content of colostrum are higher than the 63 mg per liter value previously reported

by Holmes, Spelman, Smith and Kuzmeski ('47) for normal milk produced by Percheron mares.

The colostrum from mares 1 and 3 contained approximately the same amounts of potassium, the averages being 101 mg and 100 mg per liter. The average potassium content of the colostrum produced by mare 4 was 94 mg per liter, and that for mares 2a and 2b was 80 mg per liter. Judged by these results, colostrum from Percheron mares contains significantly more potassium than their mature milk, for Holmes et al. ('47) reported 64 mg per liter for the latter.

The initial sample of colostrum from 4 of the mares contained more magnesium than the later samples. The respective means for the remaining 5 days of the 6-day periods for the 5 mares, in mg/l, were 16, 13, 16, 16 and 15 for mares 1 to 4, respectively. After the first day of lactation the magnesium content of the mares' colostrum varied from 10 mg per liter for mares 2a and 4 to 16 mg per liter for mares 1, 2b and 3. In general, the results obtained for the individual mares were quite uniform but were higher than those reported in the earlier study of the composition of mares' milk by Holmes and co-workers ('47), who found 9 mg per liter in Percheron mares' milk.

Although mares' colostrum contains two to three times as much protein and definitely more phosphorus, potassium and magnesium than does their later milk, few data are available to indicate whether or not these constituents in the colostrum are equally well or better utilized by the foal than when they occur in normal mares' milk. Frenzel ('37), from his metabolism experiments with young foals, concluded that the digestibility of colostrum during the first week of life is approximately 90%, and that the coefficient of digestibility of the constituents of mares' milk decreases gradually as the foal grows older.

SUMMARY

Thirty samples of colostrum were collected for the first 6 days of lactation from 4 normal, purebred Percheron mares.

Their ages varied from 4 to 11 years, their weights from 1600 pounds to 1900 pounds, and their lactation periods being from the first to the 6th. One mare was used for two lactation periods, her 5th and 6th.

The water content of the colostrum was similar for the different mares. The mean protein values for the 6-day period, which ranged from 3.7% to 5.4%, were markedly higher than the value of 2.3% reported for mature mares' milk.

Relatively small amounts of reduced ascorbic acid were found, i.e., from 47 mg to 66 mg per liter for mares that later produced mature milk which contained from 134 mg to 171 mg per liter.

Initial samples of colostrum contained less phosphorus than the later samples. The mean values in milligrams per liter of 86, 75, 79, 85 and 83, were higher than the 63 mg per liter obtained in an earlier study of mature milk.

The mean values for potassium for the different mares were 101 mg, 80 mg, 80 mg, 100 mg, and 94 mg per liter as compared with 64 mg per liter for mature milk.

The various samples of colostrum contained similar amounts of magnesium. The mean values were significantly higher than those previously obtained for milk produced at the end of the first month of lactation.

Values obtained for colostrum produced during two lactation periods of the same mare were in close agreement.

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